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Sugar Absorption and Surface Carbohydrase Activity of *Peltigera polydactyla* (Neck.) Hoffm.

BY

J. L. HARLEY and D. C. SMITH

(*University Department of Botany, Oxford*)

With fifteen Figures in the Text

ABSTRACT

A method suitable for the study of the physiology of absorption of substances from solution and of respiration of *Peltigera polydactyla* was elaborated making use of discs of tissue which could be floated on solutions. The rate of absorption of hexose sugars by the discs was sensitive to temperature, the presence of inhibitors, such as sodium fluoride and silver nitrate, but relatively insensitive to hydrogen ion concentration. Glucose was absorbed preferentially to fructose from equimolar solutions. Absorption of sugar from sucrose solutions involved inversion by an enzyme system held on the cell surface. This system was relatively unaffected by exposure to moderate temperature, to sodium fluoride or silver nitrate but sensitive to hydrogen ion concentration. Sucrose did not seem to be absorbed as such and the glucose was absorbed preferentially from the products of inversion. All parts of the thallus were equally effective in inversion but absorption was mainly into the algal layer. The entry into the tissues was associated with a respiratory rise.

THE experiments here described deal primarily with factors affecting sugar uptake by *Peltigera polydactyla.* Certain comparative experiments are also included which suggest that the processes described are of general application to many lichens and are related to similar processes in other kinds of tissue. Previous work on lichen physiology may be grouped under two headings: the study of the isolated fungal and algal components in pure culture and the performing of simple physiological experiments during the course of ecological studies in attempts to solve *ad hoc* problems. In these latter experiments it has often been found that lichen material is exceedingly variable so that agreement between replicates is difficult to achieve (Fraymouth, 1928; Smyth, 1934).

It cannot be too strongly emphasized that with lichens, as well as other symbiotic material, a study of the physiology of the symbiotic structures as functional entities is essential. A satisfactory knowledge of their fundamental physiological properties is the only safe background to work on ecological problems. So called aut-ecological studies of these or indeed of any plant species rely on a knowledge of physiology which is too frequently achieved by guesswork or by analogy with those organisms which have been studied in detail. If an assessment of what is known about lichen physiology is made, it cannot but be agreed that the known is a small part of what is believed, and that the explanations of ecological distribution are usually pure surmise.

These experiments were initiated particularly to obtain information concerning sampling difficulties and it was therefore decided to study some

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important process for which there were satisfactory analytical techniques. As was expected, sampling procedures of a simple kind giving adequate replication were evolved without difficulty. The sampling of *Peltigera* thalli and their preparation for experiment presented similar superable problems to the sampling of any other material grown under natural conditions; and with the same ease, samplings of other lichens were made. The study of sugar absorption by lichen tissue gave results of general interest because of comparisons which could be made with fungal tissues, leaf tissue and root tissue amongst plants, and with a variety of animal tissues.

THE PLANT MATERIAL AND SAMPLING METHODS

Thalli of *Peltigera polydactyla* (Neck.) Hoffm. were obtained from one locality only, at Hurst Hill, near Oxford. Care was taken to ensure that it was never confused with *P. horizontalis* (L.) Baumg. which although similar in sterile form is rare in this habitat. Thallus lobes, 1·5–5 cm. broad and 2–6 cm. long, were collected on the days when required between 9 a.m. and 10 a.m. Smaller lobes were never collected and at most only two lobes were removed from any one plant. The material was therefore representative of the large lobes of the whole population.

The lobes were washed in tap water and the rhizines to which dirt adhered were cut off. Discs 7 mm. in diameter were cut by means of a tempered silver-steel blanking punch, which delivered them into a Petri-dish of distilled water. After two further washes in distilled water, the discs were counted into samples of 30 for uptake experiments or 25 for respiratory experiments. Damaged or discoloured discs were rejected. Each sample was again washed in sterile distilled water and the discs floated upon their medullary faces in Petri-dishes of sterile aerated distilled water for 12–16 hours before use.

The material so prepared consists of discs having a dark olive-green upper surface and a white-veined lower surface bearing occasional rhizines. In section three anatomical regions are present. (Fig. 1.)

1. The upper cortex, 24–34 μ thick, consisting of fungal pseudoparenchyma.
2. An algal layer, 20–50 μ thick, containing small closely packed bundles of Nostoc filaments separated by thin-walled hyphae.
3. A medulla of very variable thickness, 50–1000 μ of loosely interwoven thick-walled hyphae. This great variation in thickness is due largely to the veins which form raised ridges.

In some experiments, the behaviour of the medulla was compared with that of the two upper zones. Discs were dissected into two tissue systems, upper cortex and algal zone, and medulla. The method used was as follows. Each disc was cut into four quadrants of approximately equal size which were placed upper side down in a drop of water on a slide under a dissecting microscope. An incision was made with a sharp scalpel immediately above the algal layer (Fig. 1). At an acute corner the medulla layer was lifted up and gently separated with a scalpel from the underlying alga-containing tissues.

The two parts which were approximately equal in dry weight were immediately floated on sterile water.

The labour of dissection was minimized by using only those discs of a convenient thickness, which comprised about one-third of any collection. Such 'dissectible' discs were heavier and thicker in medulla than the remainder in the ratios of 7:5 and 2:1 respectively.

The operation of dissection introduced two additional manipulations; quartering of the discs and separation of the tissues, as well as the selection of particularly thick discs. The effects of these were estimated by the control values given in appropriate tables.

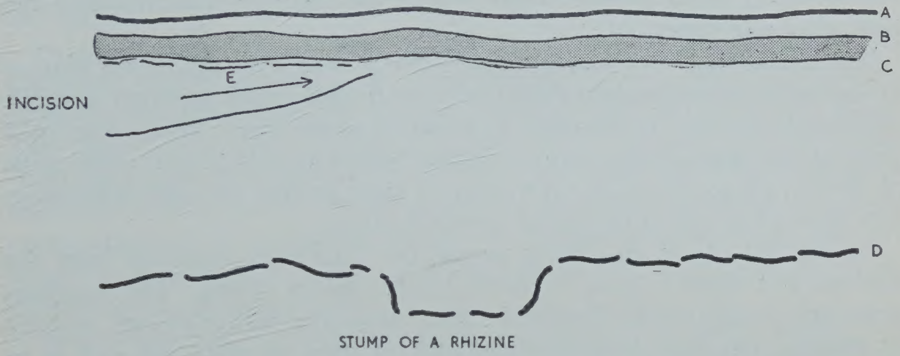

FIG. 1. Diagram showing the arrangement of the tissues in the thallus of *Peltigera*, and the plane of the incision made in separating the algal and fungal zones.

A–B Upper cortex.
B–C Algal layer.
C–D Medulla.
E Fragments of the medulla attached to the algal layer.

The sampling methods here adopted result in the selection of thallus lobes falling within a given size range and conforming to a given standard of health and physical condition as noted by colour and absence of damage. Within the material so defined there was a seasonal variation and also selection of material for the special purpose of dissection. These points have been borne in mind in interpreting the results and are analysed as far as possible in the presentation of results.

The results are expressed most frequently in terms of samples of 30 discs. This is effectively on a surface area basis. For some purposes dry weights were estimated. It was found that the material came to constant weight when kept at 75° C. for 24 hours, and since this drying method avoided charring, it was adopted.

The absorption of sugars resulted in an increase of dry weight, so for many experiments a series of 2 or 3 samples was used to estimate 'control dry weight'. The 'control dry weight' showed a slight seasonal variation which ran parallel with other seasonal changes.

EXPERIMENTAL PROCEDURE

To study sugar absorption, discs or dissected quarter-discs were floated on their medullary surfaces on sugar solutions in small Petri-dishes. Each sample of 30 was floated on 5 ml. of solution in a dish 6 cm. in diameter. Owing to the presence of air spaces surrounded by unwettable crystals of 'lichen-acids' the discs remained buoyant during the whole period of experimentation, 36–84 hours from the initial cutting. The period over which sugar uptake was studied was usually 24 hours ± 10 minutes at a temperature of 25° C. ± 0·5° C. in the dark. For most experiments the medium was aerated before use but the effect of the greater oxygen supply was small provided the discs had their algal side uppermost.

The media used were simple sugar solutions 10 or 20 g. per litre of glucose or sucrose (analytical reagent grade) or 'pure' fructose made up either in sterile demineralized water or sterilized by filtration as solutions. Where control of pH was necessary, phosphate or phthalate buffers were employed at strengths of M/100, M/50, or M/25. No effect of these buffers on sugar absorption was detected except in respect of their pH values.

At the end of the absorption period the discs were removed from the medium and washed thrice in distilled water before drying. The washings were used to wash out the Petri-dish into a volumetric flask of 100 ml. capacity or greater. The final dilution of the 5 ml. of sugar medium was such as to give a maximum sugar content of 0·5 mg. per ml. for analysis by the method described below.

The errors involved in recovery of the medium from the Petri-dishes were tested by a 10-minute flotation of 30 discs on 5 ml. samples of media. The mean of six replicate estimates was 99·8 per cent. recovery with a range of 99·4 per cent.–100·2 per cent.

The method of estimating total reducing sugars in solution was based upon that of Somogyi (1945). 5 ml. aliquots of the unknown were employed and on each day the value of the thiosulphate constant was determined using an accurately weighed sample of dry glucose. The values obtained were between 0·123 and 0·126 for N/200 thiosulphate using N/40 iodate.

Sucrose was estimated as reducing sugar after acid hydrolysis. To 25 ml. of sucrose 2·5 ml. of 10 per cent. H_2SO_4 was added and the mixture kept in a boiling-water bath for 25 minutes. After cooling on ice, the acid was neutralized with solid sodium carbonate. After dilution to suitable volume, 5 ml. aliquots were used to estimate reducing sugar.

Fructose was estimated in the presence of glucose by the method of Van der Plank (1936). This involves the destruction of glucose by alkaline hypoiodite followed by reducing sugar estimates by the Somogyi method. Two small modifications of procedure were used. Glucose destruction was carried out at 18° C. for 10 minutes instead of at 1° C. for 2 hours, and volumes of 10 ml. instead of 5 ml. were employed. Using pure sugars it was found that destruction was virtually complete after 7 minutes and that only a small

(2 per cent.) loss of fructose occurred in the 10-minute period. Solutions of invert sugars with or without added glucose, when analysed either directly as total reducing sugars or after glucose destruction, agreed within 1 per cent. The standard error of a single estimate of the fructose content of medium containing 50 mg./5 ml. of fructose mixed with glucose and sucrose was 0·3 mg. An analysis of errors involved in other sugar estimates based upon a large number of estimations is shown in Table I.

Glucose present in mixed solutions was estimated by difference. Consequently the values obtained are liable to the standard errors of both the estimation of total reducing sugars and the estimation of fructose. More attention must be paid to fructose estimates than glucose estimates in assessing the results.

In all sugar-feeding experiments the initial content of the medium was estimated on three replicate samples which had been treated exactly as the experimental samples except that no plant material was floated upon them. The results of analysis of uptake of sugars into the plant material and of reducing sugars appearing in the medium are expressed in the form—mg. sugar/30 discs/24 hours. Where sucrose was used, total sucrose utilization represents the total disappearance of sucrose from the solution (i.e. uptake of sugar+reducing sugar appearing in the medium) and is expressed as mg. sucrose unless otherwise stated.

Respiration rate was measured either by standard Warburg procedures or by use of Pettenkofer apparatus. In the Warburg experiments 25 discs and a total volume of 2·5 ml. of fluid were used in each flask. R.Q. estimates were made with replicate samples with and without KOH as an absorbent of CO_2. For estimation of CO_2 emission with the Pettenkofer apparatus, larger samples of discs were floated on sugar solution on Petri-dishes which were kept inside sealed glass bell-jars and immersed in a water bath. In all respiratory experiments the material was kept in the dark to eliminate photosynthesis.

ASSESSMENT OF ERROR AND VARIATION IN THE MATERIAL AND METHODS

Coefficients of variation of most of the measurements made are given in Table I, which indicates total variation including variation of the material as well as error in analysis. Other comparisons of a similar kind are given in the text.

One important source of error in estimation of sugar absorption by *Peltigera* discs, which has been eliminated in the methods used, depends upon the manner of their flotation on solutions. Table II compares the relative absorption rates when discs are floated on their cortical and medullary surfaces. The uptake is reduced when the upper (cortical) side is kept below the liquid. It will be shown later that the algal layer is very active in absorption and the reduction in rate may be due to a decrease in its oxygen supply when it is submerged. Weatherly (1953) has shown with *Atropa belladonna* that sugar uptake is inhibited in the dark by lack of O_2. Table III shows that discs floated on sugars on their upper (cortical) surfaces but illuminated from below have absorption rates which are greater than those of unilluminated discs but equal to those of discs floated in a normal manner.

TABLE I

Coefficients of Variation

(C.V.) = standard deviation × 100%. Any deviation which is 2 × C.V. from the mean is significant at the level $P = 0{\cdot}05$

Factor	Mean value	Standard deviation	Coefficient of variation (*standard deviation × 100%*) mean
Uptake of glucose (mg./30 discs/24 hours)	20·9 mg.	1·06 mg.	5·1%
Uptake from sucrose (mg./30 discs/24 hours)	21·5 ,,	1·61 ,,	7·5%
Reducing sugars appearing in sucrose media (mg./30 discs/24 hours) .	35·1 ,,	2·5 ,,	7·1%
Total sucrose utilization (mg./30 discs/24 hours)	56·6 ,,	3·0 ,,	5·3%
Final dry weight of samples on glucose media (sample size—30 discs) .	88·9 ,,	3·7 ,,	4·2%
Final dry weight of samples on sucrose media (sample size = 30 discs) .	87·7 ,,	2·65 ,,	3·0%
Dry weight of control samples (sample size = 30 discs)	69·8 ,,	2·6 ,,	3·7%
Respiration rate expressed as O_2 uptake/30 discs/hour (sample size = 25 discs)	76μl	3μl	3·9%

TABLE II

The Effect on Uptake, &c., of Floating Samples on the Upper (Cortical) Surfaces instead of on their Lower (Medullary) Surfaces

Expt. No. and date	No. 37 (23/7/54)			No. 4 (20/4/53)	No. 26 (17/3/54)
Sugar and concentration	2% Sucrose			2% Glucose	1% Glucose
	Uptake	Reducing sugars in medium	Total sucrose utilization	Uptake	Uptake
Samples floated on lower (medullary) surface	19·4 ± 0·7	30·5 ± 1·1	49·9 ± 1·3	24·5 ± 0·6	23·2 ± 1·1
Samples floated on upper (cortical) surface	13·0 ± 1·1	43·5 ± 1·8	56·5 ± 2·1	20·0 ± 0·6	19·0 ± 1·1

EXPERIMENTAL RESULTS

Table IV illustrates the order of magnitude of the rate of sugar absorption from glucose, sucrose, and fructose solutions. In this range of experiments, the quantities absorbed by 30 discs in 24 hours lay between 17 and 27 mg. Of this, some 60–90 per cent. appeared as increase in the dry weight of the discs and the remainder was lost presumably in respiratory CO_2 and perhaps other end-products.

TABLE III

The Effect of Light on Uptake by Discs Floated Algal Layer Down

Values mg./30 discs/24 hours at 25° C., pH 5·6 M/100 phthalate

Medium	Treatment during flotation	Orientation of discs during flotation	Uptake	Reducing sugars appearing in sucrose media	Final dry weight
2% sucrose	Illuminated	Discs floated on cortical surface	26·9	56·5	99·9
		,,	24·7	58·1	96·0
	Not illuminated	,,	18·0	65·4	88·5
		Discs floated on medullary surface	24·9	56·8	95·5
Standard errors of 1 reading (sucrose):			±1·6	±2·5	±2·7
1% glucose	Illuminated	Discs floated on cortical surface	27·0	—	99·0
		,,	26·1	—	98·2
	Not illuminated	,,	19·0	—	89·9
		Discs floated on medullary surface	25·2	—	98·2
Standard errors of 1 reading (glucose):			±1·1	—	±3·7

TABLE IV

Increase in Dry Weight with Uptake of Sugar

This table shows the difference between the mean final dry weights of samples floated on three types of sugar media and the mean initial dry weight of control samples. In all cases, flotation = 24 hours at 25% C.

Medium	Expt. No. and date	Mean initial (control) dry weight (mg./30 discs)	Mean final dry weight (mg./30 discs)	Increase in mean weight (mg./30 discs)	Mean uptake mg./30 discs/24 hours	Increase in mean weight as percentage of uptake
2% Sucrose	8 (4/6/53)	81·5±1·8	99·0±1·9	17·5	25·1±1·1	68%
	20 (19/1/54)	64·1±1·8	83·3±1·9	17·2	21·9±1·1	79%
	25 (9/3/54)	68·6±1·3	90·3±1·9	21·7	25·5±1·1	85%
	26 (17/3/54)	73·5±1·5	97·5±1·9	24·0	26·2±1·1	92%
	27 (29/3/54)	75·8±1·8	94·3±1·9	18·5	25·8±1·1	72%
	28 (4/5/54)	86·3±1·8	102·6±1·5	16·3	24·3±0·9	67%
	29 (17/5/54)	75·5±1·8	98·3±1·9	21·8	24·9±1·1	88%
	37 (23/7/54)	57·8±1·5	73·6±1·2	15·8	19·4±0·7	82%
1% Glucose	8 (4/6/53)	81·5±1·8	95·2±2·6	13·7	21·9±0·8	63%
	25 (9/3/54)	68·6±1·3	88·9±2·6	20·3	21·5±0·8	94%
	26 (17/3/54)	73·5±1·5	94·2±2·6	20·7	26·9±0·8	77%
	28 (4/5/54)	86·3±1·8	100·7±2·1	14·4	23·0±0·8	63%
1% Fructose	28 (4/5/54)	86·3±1·8	101·2±2·1	14·9	20·4±0·8	73%
	(23/7/54)	57·8±1·5	69·9±2·6	12·1	16·0±0·8	76%

Over the period of 20 months, during which experiments were made, there was a slight seasonal variation in the rate of uptake which ran parallel to variation in dry weight. Samples gave maximum values for both uptake and dry weight in spring, and minimum during autumn and early winter.

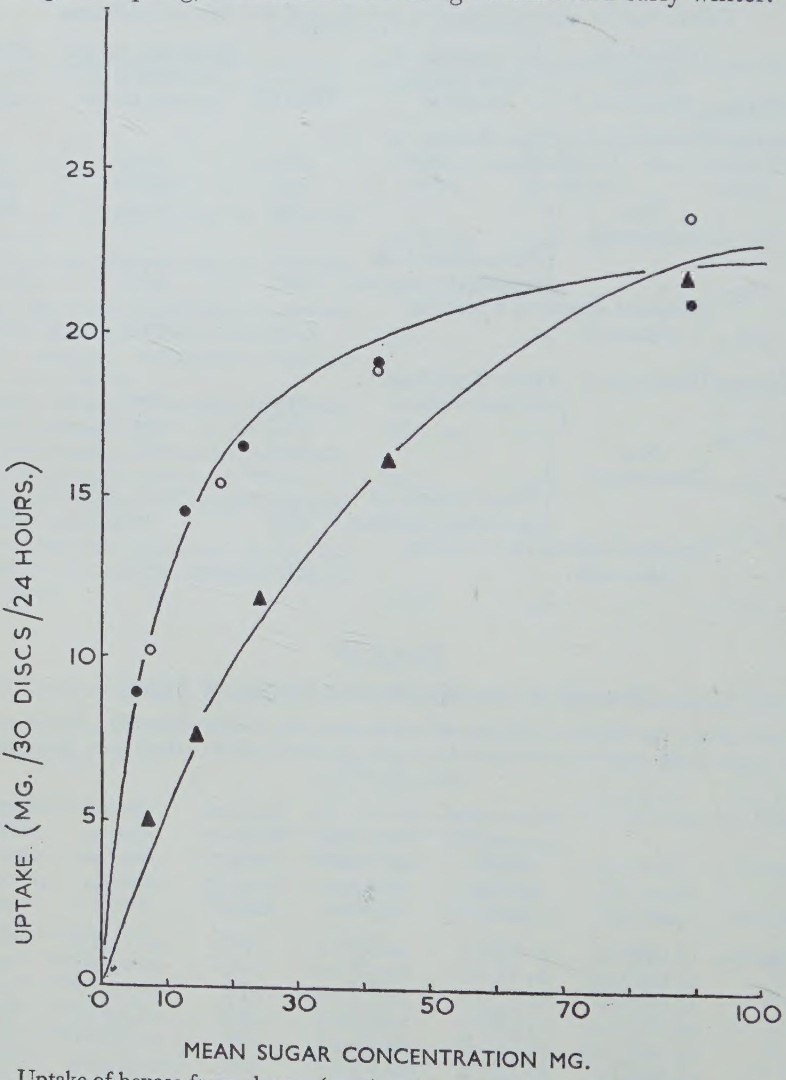

FIG. 2. Uptake of hexose from glucose (● ○) and fructose (▲) media of different concentrations. Mg. of sugar absorbed in 24 hours by 30 discs at 25° C. plotted against mean sugar concentration.

The factors affecting absorption rate have been studied in some detail. The effects of concentration upon absorption of monosaccharides will be described first, since the additional feature of sucrose breakdown in solution makes the absorption of this sugar more complicated.

Fig. 2 shows the effect of concentration on absorption of glucose and fructose. Here uptake is plotted against the mean external concentration during the uptake period. Both curves approximate to rectangular hyperbolae but of strikingly different form. The asymptote for glucose curve based on two experiments is 23·8–24·9 mg. per 30 discs in 24 hours with a half maximum value at 8·8–10·6 mg. per 5 ml. (10 mM. approximately). The asymptote for fructose is 36·5 mg. per 30 discs in 24 hours with a half maximum value of 50·1 mg. per 5 ml. (about 50 mM.). These results suggest that the process of absorption of monosaccharides is not purely a diffusion process but one that may involve a reaction of sugar with a carrier or enzyme which selects glucose more rapidly than fructose from dilute solutions.

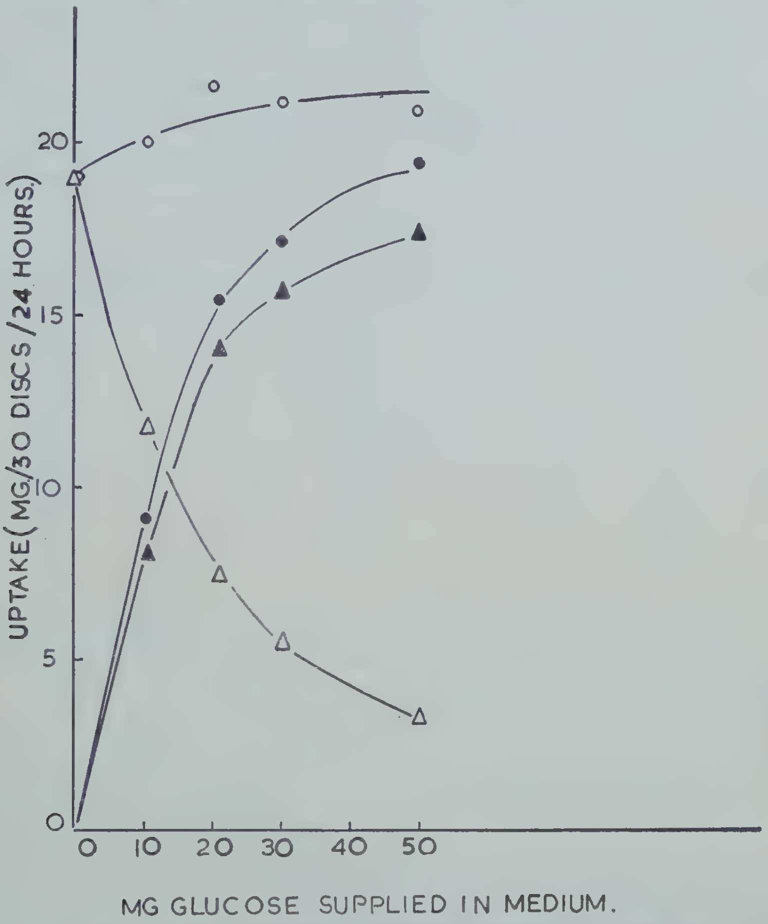

FIG. 3. The uptake of hexose from fructose solution containing 51·5 mg. per 5 ml. in the presence of glucose.

–○– Total sugars absorbed.
–△– Fructose absorbed.
–▲– Glucose absorbed.
–●– Uptake of glucose in absence of fructose.

This point is further emphasized by uptake from mixed solutions. In Fig. 3

an example is given of uptake from a fructose solution of 1 per cent. (50 mM.) to which increasing amounts of glucose were added. In the presence of glucose no very great increase of total absorption occurred although the total external concentration of reducing sugars was greater. Indeed, the presence of glucose caused a very great diminution of fructose absorption, so that when the sugars were present in approximately equimolar concentration, the total sugar uptake was only increased by some 10 per cent. The comparison of uptake of glucose from a pure solution with one in which fructose also was present shows that glucose uptake is only diminished by about 10 per cent. by the presence of fructose.

TABLE V

Illustrating the Mean Rate of Absorption of Sugar and the Release of Reducing Sugars by Samples of 30 discs in 24 hours. Sucrose Concentrations 2%

Expt. No.	Uptake Sucrose/30 discs/24 hours	Reducing sugar
8	25·1	27·2
20	21·9	43·5
25	25·5	38·1
26	26·2	56·3
27	25·8	—
28	24·3	40·8
29	24·9	—
37	19·4	30·4

In all the experiments upon sucrose absorption by *Peltigera*, reducing sugars appeared in the external solution. The quantities involved in the experiments shown in Table IV are set out in Table V.

A more complete picture of this process is shown in Fig. 4. In this experiment a large sample of 300 discs was floated on 50 ml. of 2 per cent. sucrose in a Petri-dish 14 cm. diameter at 25° C. At intervals 2 ml. samples were withdrawn with sterile pipettes for analysis of reducing sugars and total sugar. The percentage of the total sugar in the medium, which consisted of sucrose, fell from 100 per cent. to 7·5 per cent. in 36 hours. During that period reducing sugars increased in quantity rapidly at first and more slowly later. Over the whole 72 hours a steady removal of sugar by the plant material occurred amounting to some 200 mg. in total.

Chromatographic analysis of the sugars present in the medium during experiments of this kind, using the method of Partridge (1948), showed the presence of glucose, fructose, and sucrose and no others. It seems likely therefore that the reducing sugars are formed from sucrose by inversion and not from other sources. During this process of inversion of sucrose no signs of a free sucrose-splitting enzyme were found in the medium. During the experiment described in Fig. 4, 2 ml. samples of the medium were withdrawn simultaneously with the analytical samples and incubated at 25° C. for 30 hours before analysis. The results are shown as replicate points at 6, 12, and 18 hours on the graph. As can be seen they do not depart significantly from the results of immediate analysis. This is not only good evidence of the absence of a free enzyme but also of the absence of contaminating micro-organisms in the medium. It does not wholly prove the absence of surface contaminants.

Repetition of this kind of experiment with prolonged incubation, thorough washing of discs, and the testing of washing water for sucrose inversion, together with microscopic inspection of the plant material and solutions, has failed to give evidence of the presence of contaminants. It may be safely assumed that the conversion of sucrose to glucose and fructose is the result of a surface enzyme activity and is not due to secretion of free enzymes or to contaminating organisms.

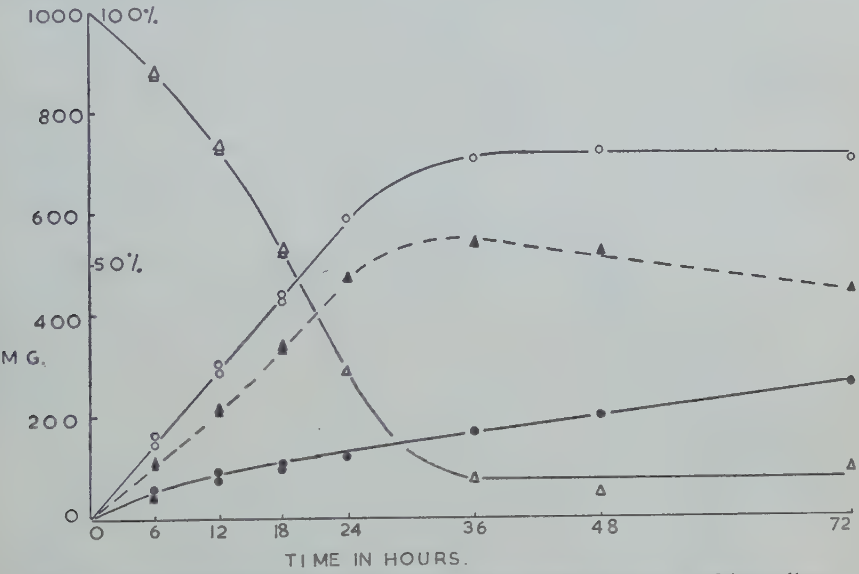

FIG. 4. The release of hexoses from 50 ml. of 2% sucrose by 300 *Peltigera* discs.
–△– Percentage of initial sucrose remaining. –○– Total sucrose utilized in mg.
–▲– Reducing sugar in the medium. –●– Sugar absorbed.
All results as mg. of sucrose.

The effect of the concentration of sucrose in the medium upon the absorption rate and upon the production of reducing sugars is shown in Fig. 5, where the absorption rate of glucose is also shown over a similar range of concentration (wt/vol.). Up to a concentration of 1 per cent. sugar the curves are almost coincident, above this value sucrose uptake falls below glucose. There is no simple proportionality between sucrose uptake and total reducing sugars, nor is the quantity of reducing sugars released equal to the amount of sugar absorbed. A mechanism involving the splitting of sucrose at the cell surface as part of the mechanism of uptake, so that one hexose moiety is absorbed and the other released, is clearly inapplicable in simple form.

The nature of the reducing sugars appearing in sucrose media

In Fig. 6 are the results of an experiment in which the release of hexoses from sucrose was studied quantitatively. In all cases the quantity of fructose found exceeded half the quantity of total reducing sugars. In media of low initial sucrose content almost all the free reducing sugar was fructose. The glucose uptake greatly exceeded the fructose uptake so that from concentrations greater than 25 mg./5 ml. the ratio of glucose/fructose uptake was apparently 2·5.

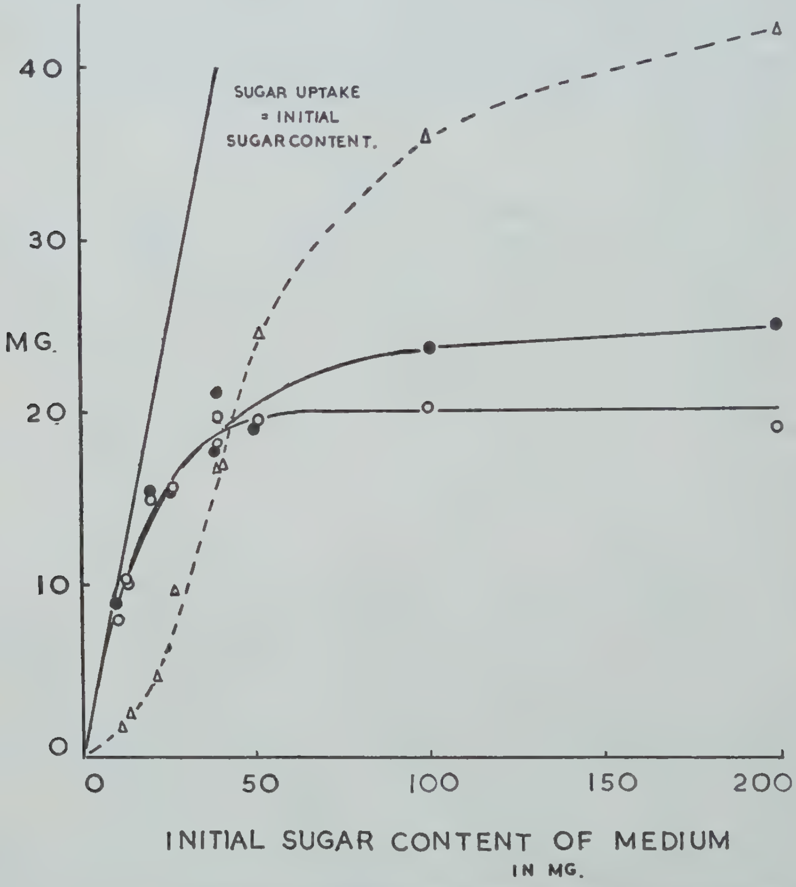

FIG. 5. The effect of initial sugar concentration upon sugar uptake from 5 ml. of medium by 30 discs in 24 hours.

– ●– Mg. glucose absorbed.
–○– Mg. sucrose absorbed.
– △– Reducing sugar released as mg. sucrose.

This result falls exactly in line with that described previously, showing the selection of glucose from mixed solutions of glucose and fructose. This question was further studied by a comparison of the uptake of sugars from a 2 per cent. sucrose solution and a 2 per cent. equimolar mixture of glucose and fructose.

The results of a series of experiments of this kind are shown in Table VI. In each experiment the total uptakes were of a similar order and the uptake

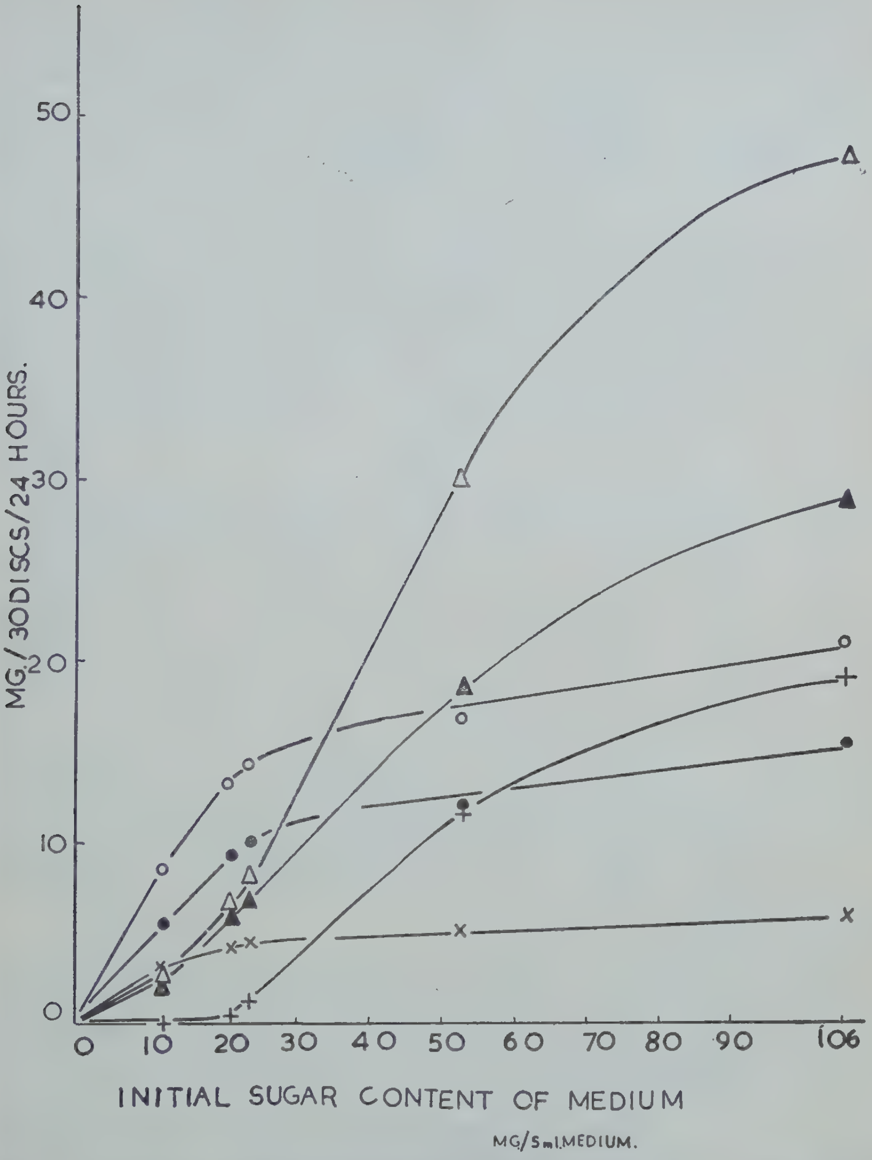

Fig. 6. The effect of the initial sucrose content of medium in mg./5 ml. of solution upon the amounts of glucose and fructose lost in uptake and released as free reducing sugars. Results in glucose equivalents.

–○– Total uptake.	–△– Total released.
–●– Glucose component of uptake.	–▲– Fructose released.
–×– Fructose component of uptake.	–+– Glucose released.

of glucose exceeded that of fructose by a considerable margin. The amount of fructose lost from the sucrose media exceeded that lost from the mixture. These results are explicable if it is assumed that uptake from sucrose solution is mainly an uptake of reducing sugars of which glucose is preferentially absorbed. During the initial phase of absorption, the concentration of hexoses will be low but will increase with time (Fig. 4). Hence, at first, absorption will be rate-limited by hexose supply, and both glucose and fructose will be absorbed. As more hexoses become available, glucose will be absorbed in more and more preferential quantities. Although this is no proof that sucrose is not absorbed as such, it is clear that *Peltigera* discs absorb glucose preferentially to both fructose and sucrose, and that a surface enzyme, not directly involved in absorption, causes a breakdown of sucrose to glucose and fructose.

TABLE VI

Relative Amounts of Glucose and Fructose Absorbed from a 2% Sucrose and a 2% Equimolecular Mixture of Glucose and Fructose

Uptake values are for individual samples and are expressed as mg. reducing sugar/30 discs/24 hours, media at pH 5·4–5·6 (M/100 phthalate). Temperature 25° C.

	Uptake from 2% sucrose media				Uptake from a 2% equimolecular glucose-fructose mixture			
Expt. No. and date	Total uptake	Fructose component of uptake	Glucose component of uptake	Fructose component as percentage total uptake	Total uptake	Fructose uptake	Glucose uptake	Fructose uptake as a percentage total uptake
28 (4/5/54)	23·9	9·3	14·6	39%	26·1	6·2	19·9	24%
	26·3	8·2	18·1	31%	25·7	6·7	19·0	26%
	26·1	8·3	17·8	32%	26·4	6·6	19·8	25%
29 (17/5/54)	26·2	6·3	19·9	24%	26·3	5·3	21·0	20%
	26·4	7·3	19·1	28%	25·7	5·9	19·8	23%
30 (29/5/54)	22·7	7·3	15·4	32%	22·9	5·0	17·9	22%
31 (9/6/54)	19·3	4·5	14·8	23%	18·6	3·3	15·3	18%

The effect of temperature on absorption

The selection of glucose in preference to fructose from equimolar solutions, or from sucrose, suggests that absorption of sugars is linked with metabolic processes or, in any event, is dependent on chemical rather than physical processes. This is further upheld by the fact that the effects of temperature upon the rate of absorption are greater than that expected from a process limited in rate by diffusion. The results of three experiments are shown in Table VII, where the quantities of sugar absorbed and sucrose inverted were estimated for two temperatures, 2 or 2·5° C., and 24·5 or 25° C.

The effect of pH on absorption

The uptake of sugars would not be expected to be as sensitive to hydrogen ion concentration as that of ionized substances, but the inversion of sucrose, if due to a surface enzyme, would depend on pH. The pH dependence of enzyme systems *in vivo* has been used as evidence of their situation

TABLE VII

Temperature Coefficients (Q_{10}) of Uptakes from Sucrose and Glucose, appearance of Reducing Sugars in Sucrose Media, and Total Sucrose Utilization

Each value in the Table is a mean of two readings.

Expt. no. and date	Medium	Temperature interval	Uptakes (mg./30 discs/24 hours)			Reducing sugars appearing in sucrose media (mg./30 discs/24 hours)			Total sucrose utilization (mg./30 discs/24 hours)		
			Low temp.	High temp.	Q_{10}	Low temp.	High temp.	Q_{10}	Low temp.	High temp.	Q_{10}
2 (27/2/53)	2% Glucose (in water)	2°–25° C.	3·7	25·3	2·31	—	—	—	—	—	—
14 (10/10/53)	2% Sucrose pH 6·4 (M/100 phthalate)	2·5°–25° C.	5·4	18·0	1·71	5·0	16·2	1·69	10·4	34·2	1·69
20 A (19/1/54)	2% Sucrose pH 4·7 (M/100 KH_2PO_4)	2·5°–24·5° C.	5·6	21·9	1·86	19·3	50·0	1·54	24·0	71·9	1·62

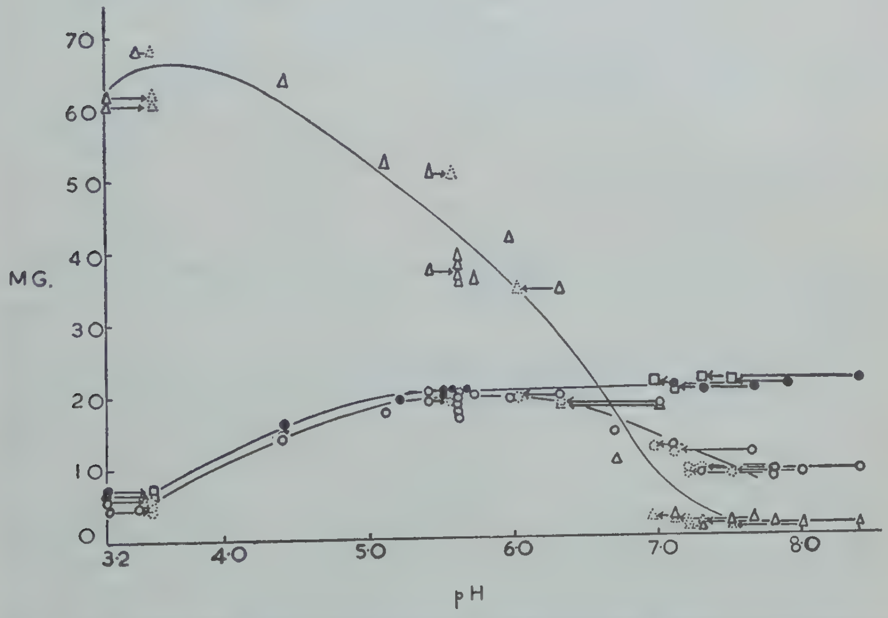

FIG. 7. The effect of the pH of the medium on uptake of glucose, uptake from sucrose, and appearance of reducing sugar in sucrose media. The range of pH change given and each case concentration 2%.

□– ● Uptake of glucose in mg.
○– ○ Uptake of sucrose in mg.
△–△ Reducing sugar as mg. sucrose.

on the surface of tissues (Rothstein, 1954). A number of experiments on this point have been carried out using as buffers phosphate and phthalate. At the limits of the ranges of these buffers some change of pH was observed during the experimental period, so the range of pH has been given in the figures and tables.

Fig. 7 is compiled from the results of seven experiments made during

October and December, consequently a slightly greater variation than usual, due to season, is present. In each case the experimental points were controlled by analyses of samples of media kept at the same pH values but in which no lichen discs were placed. By this means a slight acid inversion of sucrose at pH 3·2 has been corrected. Similar rates of uptakes from glucose and sucrose were observed over the range 3·2–6·0, but above this range the curves diverge. The depression of sucrose uptake above pH 6·0 is explicable in terms of the effect of pH on the enzymic production of glucose and fructose from sucrose. The curve for reducing sugar present in the medium has a maximum value at about pH 3·6–4·0 and falls sharply above this.

The inhibition of uptake from sucrose at high pH values is completely reversible. As shown in Table VIII, the normal rate of absorption is regained when discs, kept at pH 8·0, are returned to pH 5·5. This is strong evidence that the main absorption of sugar from sucrose is in the form of reducing sugar.

TABLE VIII

The Uptake of Sucrose by Discs Floated on M/50 Phosphate Buffer at pH 5·5 and pH 8·0

Results as means of two readings expressed as mg. sucrose per 30 discs in 24 hours. Temperature 25° C. Expt. 28/1/54.

Treatment	1st period uptake	2nd period uptake
Floated at pH 5·5 then at pH 8·0 . . .	19·0	8·3
Floated at pH 8·0 then at pH 5·5 . . .	8·8	18·6

The inhibition of uptake in the lower range of pH is common to both glucose and sucrose and cannot be ascribed to an effect on the inversion system which has here a high rate of activity. Indeed, the greatest rate of total sucrose utilization is found between pH 4·2 and pH 4·6. The inhibition of uptake is not completely reversible as is shown in Table IX. A 16-hour pre-treatment at pH 3·2 causes a diminution of about 21 per cent. during a subsequent 24-hour period of uptake from sucrose. Total sucrose utilization is unaffected by the pre-treatment. This inhibition by low pH is associated with a permanent colour change from green to mauve of the algal layer and may be due to damage of the algal cells which are extremely active in uptake.

The role of inversion in absorption from sucrose media

In Fig. 8 the rate of sugar uptake from sucrose is plotted against total sucrose utilization. The figures are drawn from two experiments of a different kind; one in which uptake and inversion were studied in a range of sugar concentrations, at a constant pH, and one in which uptake and inversion were estimated in 2 per cent. sucrose in a range of pH values. The results of both types give graphs which are apparently coincident, and approximate to rectangular hyperbolae. Hence the uptake from sucrose solution is similarly related to availability of reducing sugars as are glucose and fructose uptake to external concentration.

From this it should follow that any factor which diminishes absorption from a sucrose solution, but does not affect the formation of reducing sugar, should result in an increased release of reducing sugar in solution. This is clearly true of the experiment shown in Table IX where pre-treatment at pH 3·2 checks uptake and stimulates reducing-sugar production to an equal degree.

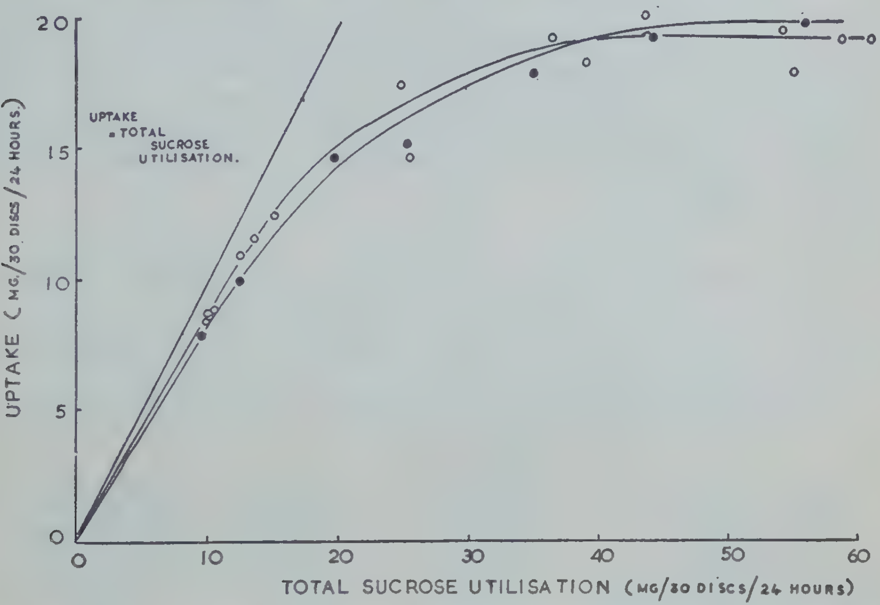

Fig. 8. Uptake of sucrose in mg. plotted against total sucrose utilization. –○– Initial sugar content constant at 100 mg./5 ml., pH varied between 5·4 and 8·4. –●– Initial sugar content varied between 10 and 100 mg./5 ml., pH constant at 5·7.

Table IX

Comparison of Rates of Sucrose Inversion and Sugar Absorption by Discs Pre-treated on Sugar-free Buffer (M/100 Phthalate) for 16 hours at pH 5·6 or pH 3·2 before being placed on 2% Sucrose

Values as mg. Sucrose/30 discs/24 hours. Temperature 25° C. Expt. 17/7/54.

pH of pre-treatment	Uptake	Reducing sugar in medium	Total sucrose utilized
5·6	20·6	47·8	68·4
3·2	16·2	53·3	69·7
Standard error of mean. 2 readings . . .	±1·1	±1·8	±2·1

A similar effect is obtained by heat treatment of *Peltigera* discs. If discs are heated at 75° C. for 24 hours, their ability to absorb sugars is abolished but they are capable of inverting sucrose. A series of pairs of disc samples were treated as described in Table X and then floated on 2 per cent. sucrose for 24 hours. Heating to 75° C. diminished or abolished uptake, but increased the rate of production of reducing sugar.

Silver nitrate has a greater inhibiting effect upon sugar absorption from sucrose solutions than upon inversion. This is in direct contrast to the results of Rheinhold (1949) who found that the reducing sugar production by carrot discs on sucrose was inhibited by 10-6 M. silver nitrate. In Fig. 9, silver nitrate at 10^{-6}–10^{-4} M. inhibits uptake of sugar whilst the amount of reducing sugars appearing in the medium is greatly stimulated. At 10^{-3} M. both uptake and inversion are almost abolished. These results confirm the view that inhibition of absorption may increase the quantity of reducing sugar released and again suggest that the two processes of absorption and inversion are essentially independent.

TABLE X

The Effect of Pre-heating Samples at 75° C. and 100° C. on their Ability to Produce Reducing Sugars in Sucrose Media

At each of the two temperatures, one pair of samples is previously air dried, and the other kept in a moist state during heating.

NB. All sugar weights as equivalent weight sucrose, media = 5 ml. 2% sucrose, pH 5·5 (M/100 phthalate). Expt. 37 (23/7/54).

Treatment of sample before flotation on sugar media	Reducing sugars appearing in sucrose media (mg./30 discs/ 24 hours)	Uptake (mg./30 discs/ 24 hours)	Total sucrose utilization (i.e. reducing sugars and uptake)
Control treatment (i.e. 18 hours on water at room temperature	29·5 27·6	19·7 20·4	49·2 48·0
18 hours' heating at 75° C. in a dry state	48·0 46·7	3·5 2·0	51·5 48·7
18 hours' heating at 75° C. in a moist state	56·2 49·9	0 0	56·2 49·9
1½ hours' heating at 100° C. in a dry state	0·1 0·3	0 0	0·1 0·3
1½ hours' heating at 100° C. in a moist state	0 0	0 0	0 0

The differential inhibition of absorption and inversion was studied in detail using sodium fluoride. This inhibitor was chosen in preference to sodium azide which was used by Mandels (1951 and 1954) since it has, unlike azide, no effect on oxidase systems. Phloridzin, used by Street and Lowe (1950) has the particular disadvantage of affecting sucrose analysis. Fig. 10 shows the effect of fluoride concentrations between 2 mM. and 40 mM. on absorption from glucose and sucrose at pH 5·6. Similar inhibitions were reached amounting to 80–90 per cent. at 40 mM. In all the subsequent experiments 25 mM. fluoride which gave an inhibition of absorption of about 80 per cent. and a slight inhibition of inversion was used.

Fig. 11 shows a comparison of sugar uptake and release of reducing sugars from a range of sucrose concentrations in the presence or absence of 25 mM. fluoride. When uptake of sugar from low concentrations is inhibited, a rise in the quantity of reducing sugars results. This is not the case in the high sucrose concentrations, where the decrease in the production of reducing sugars, due to inhibition of inversion, is quantitatively more than the decrease in their absorption in the presence of fluoride.

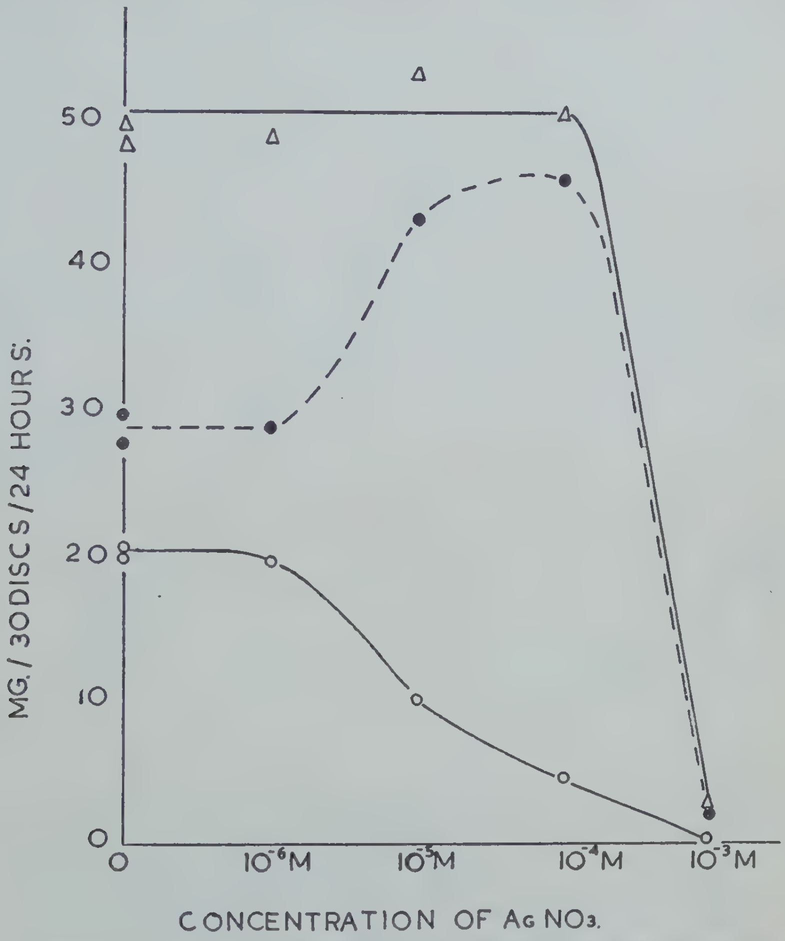

Fig. 9. The effect of AgNO$_3$ upon uptake from and inversion of sucrose. Results as mg. of sucrose. Sugar content 2%.

–○– Uptake. –●– Reducing sugar in medium. –△– Total sucrose utilized.

Although these results strongly suggest that uptake from sucrose is dominantly uptake of reducing sugars, there remains the possibility that the residual uptake in the presence of 25 mM. fluoride or at high and low pH levels is an uptake of sucrose itself. If, however, this were the case, the quantities of glucose and fructose released by inhibited discs should be nearly equal. Table

XI shows that a discrepancy between glucose and fructose still exists when discs are inhibited.

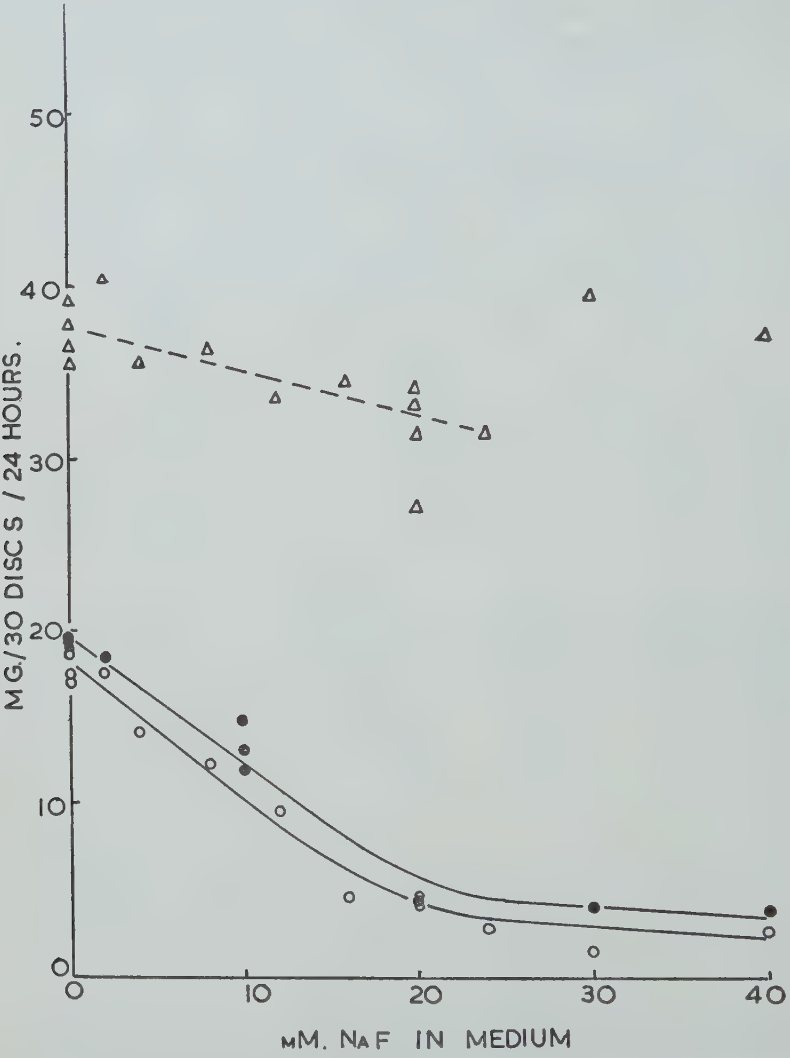

FIG. 10. The effect of NaF upon uptake from glucose and sucrose and upon release of reducing sugar for sucrose. Sugar concentration 2%.

–●– Mg. glucose absorbed.
–○– Mg. sucrose absorbed.
–△– Reducing sugar as mg. sucrose.

A variety of plant materials, carrot discs (Rheinhold, 1949), tomato roots (Street and Lowe, 1950), *Cucurbita* roots (Brown R., 1952), *Myrothecium* (Mandels, 1954), and yeast (Rothstein, 1954) have been shown to invert sucrose during uptake and to release reducing sugars. Each of these investigators has suggested a different mechanism for uptake. The present material,

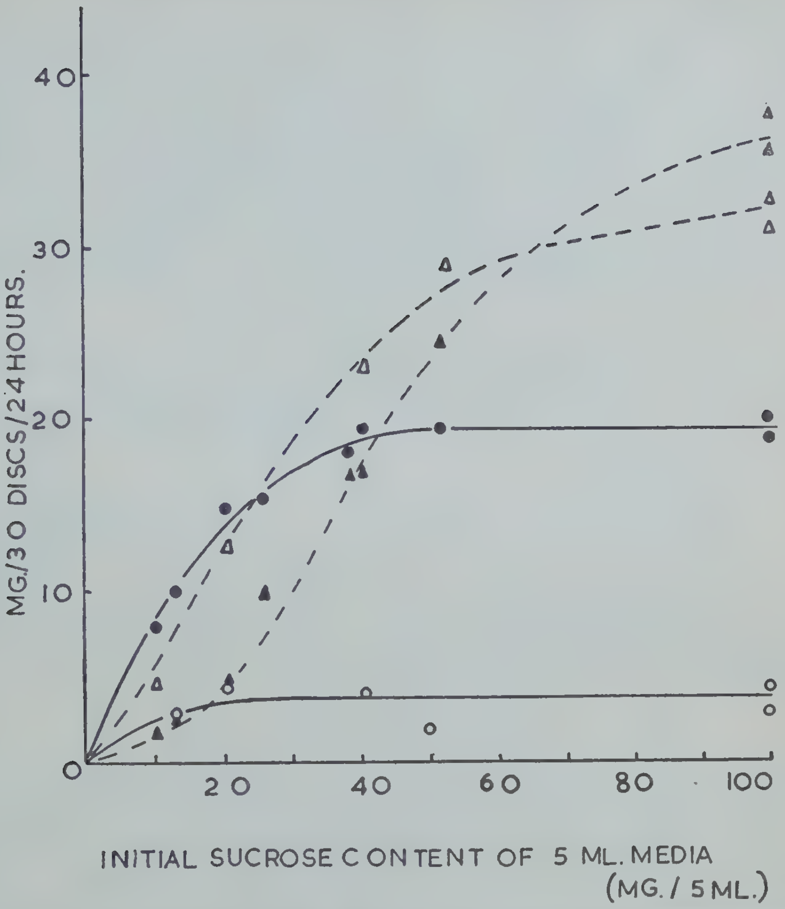

FIG. 11. Effect of 25 mM. NaF upon uptake from and inversion of sucrose in solutions of varying sugar concentration.

–○– Reducing sugar in presence of NaF. – ●– Control.
– △– Uptake in presence of NaF. – ▲– Control.

TABLE XI

Quantities of Glucose and Fructose Produced by Inhibited Discs in 24 hours at 25° C. from 2% Sucrose

Medium	Reducing sugars appearing in medium			Uptake from medium		
	Total	Fructose	Glucose	Total	Fructose	Glucose
0·4% sucrose containing M/40 NaF at pH 5·5	12·4	7·4	5·0	3·5	0·5	3·0
	12·1	7·2	5·9	4·0	0·9	3·1
2% sucrose, initial pH 3·2	42·6	22·8	19·8	5·0	1·0	4·1
	38·4	21·0	17·4	6·2	1·3	4·9
2% sucrose, initial pH 8·0	2·0	1·2	0·8	10·3	4·9	5·4
	1·8	1·7	0·1	9·6	4·0	5·6

Peltigera, seems to be again different. Certain observers (Rheinhold, 1949; Harley, McCready, and Geddes, 1954; and Brown, R., 1952) have demonstrated an increase of respiration rates during sugar absorption. Brown and Harley *et al.* have concluded that sugar absorption, like the absorption of salts, is an active process dependent on the consumption of energy. It therefore appears probable that the effect of fluoride might be indirectly through the mechanism of coupling of energy release to absorption, and to fall into line with the similar effects of azide on *Myrothecium* (Mandels, 1954) and of azide, D.N.P., and cyanide on mycorrhiza (Harley *et al.* in press).

Table XII shows the effect of glucose feeding on CO_2 emission, dry-weight increment, and sugar uptake during 24 hours. The greater part of the sugar absorbed is represented by the weight increase, although the CO_2 emission in sugar rises. Figs. 14 and 15 give examples of the results of Warburg experiments where sucrose or glucose were applied from the side arms. In each case a considerable respiratory rise was observed involving O_2 uptake and CO_2 emission. The increase of CO_2 emission exceeded the increase of O_2 uptake so that the R.Q. rose. Table XIII summarizes the relative rates during the steady states before and after sugar application. The initial rates are of the same order of magnitude as those of Scholander *et al.* (1952), Smyth (1934), and Boysen-Jensen and Muller (1929) for *Peltigera* species. The low R.Q. values before sugar application are a little lower than those found by Jumelle (1890) from several lichens. The rise in respiration rate and rise in R.Q. is similar to that reported for beech mycorrhizas when salts or sugars are applied after storage in water (Harley *et al.*, 1954 and 1956). This has been ascribed to the increase of ~P acceptors in the tissue as a result of consumption of high-energy phosphate during absorption.

TABLE XII

The Amount of CO_2 *produced by Samples of Discs during a 24-hour Flotation Period at 25° C. on Glucose and Water*

Sample size = 56 discs per sample. Values—mg./30 discs/24 hours. Experiment No. 3 (17/3/53).

	Uptake of glucose (mg./30 discs/24 hours)		Final dry weight (mg./30 discs)			CO_2 production (mg./30 discs/24 hours)		
Medium	Readings	Mean	Readings	Mean	Increase in weight	Readings	Mean	Increase in CO_2 production
2 % Glucose	14·8 15·6	15·2	73·7 76·3	75·0	10·6	5·2 6·8	6·0	3·2
Water	— —	—	63·4 65·3	64·4	—	3·3 2·4	2·8	—

Figs. 14 and 15 show the effect of fluoride in the presence or absence of glucose or sucrose. In each case a considerable respiratory stimulation, involving great increase of CO_2 emission and O_2 uptake, follows the application of fluoride, involving a rise in R.Q. There then follows a return to normal rates.

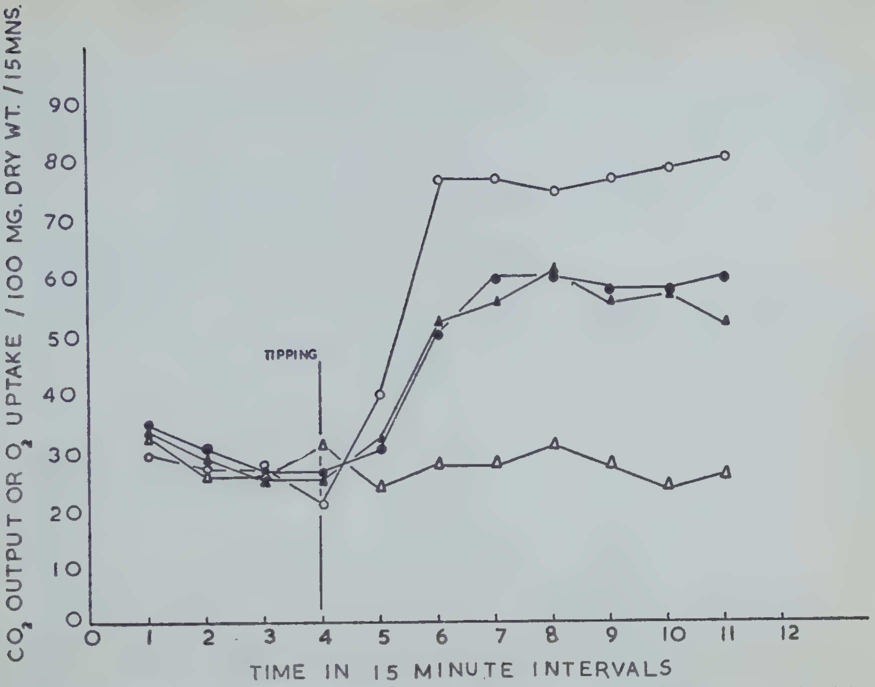

FIG. 12. The effect of 0·5% sucrose on the oxygen absorption and CO_2 emission of *Peltigera* discs in Warburg flasks.
–●– –▲– O_2 uptake. –○– CO_2 emission.
–△– O_2 uptake of control.

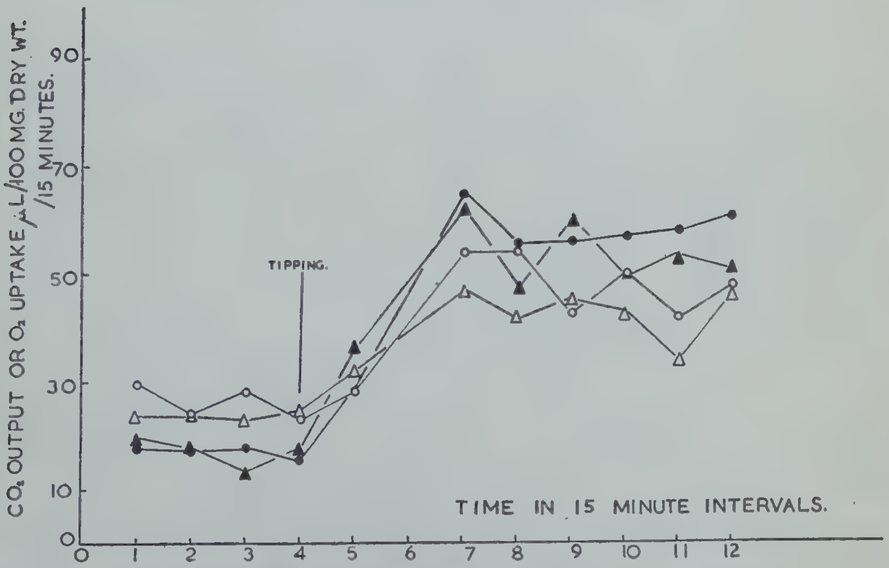

FIG. 13. The effects of 0·5% glucose and sucrose upon the oxygen absorption and CO_2 emission of *Peltigera* discs.
–△– –▲– O_2 and CO_2 of glucose samples.
–○– –●– O_2 and CO_2 of sucrose samples.

TABLE XIII

Respiration Rates and Respiratory Quotients of Samples of Discs before and after Addition of Sugars to Medium. Values from three Warburg Experiments

N.B. Rates = µl. O_2 or CO_2/100 mg. dry weight/hour at 25° C.
Sample size = 25 discs.
Sugar concentration = 0·5%, pH = 5·5 (M/100 phthalate)

Expt. No.	Sugar added to medium	Type of respiration	Rates before sugar added		Rates after sugar added		Increase in rates with addition of sugar		Final dry weight (mg./25 discs)
			Rate	R.Q.	Rate	R.Q.	Increase in rate	$\frac{CO_2 \text{ increase}}{O_2 \text{ increase}}$	
32 (16/6/54)	Sucrose	CO_2 output	103	0·89	311	1·31	208	1·71	53·9
		O_2 uptake	116		238		122		53·8
	—	CO_2 output	—	—	—	—	—	—	50·4
		O_2 uptake	113		—		—		59·3
	Sucrose	CO_2 output	—	—	—	—	—	—	57·9
		O_2 uptake	110		224		114		57·3
33 (21/6/54)	Sucrose	CO_2 output	66	0·64	231	1·23	165	1·96	59·2
		O_2 uptake	104		188		84		58·1
	Glucose	CO_2 output	52	0·55	198	1·29	146	2·48	58·5
		O_2 uptake	95		154		59		61·2
	Glucose	CO_2 output	68	0·68	210	1·28	142	2·22	57·7
		O_2 uptake	100		164		64		58·8
34 (24/6/54)	Glucose	CO_2 output	—	—	253	1·21	192	(1·7)	53·2
		O_2 uptake	—		209		130		56·9
	—	CO_2 output	61	0·69	—	—	—	—	53·4
		O_2 uptake	89		—		—		57·0
	Sucrose	CO_2 output	—	—	246	1·26	185	1·6	61·5
		O_2 uptake	—		196		107		55·8

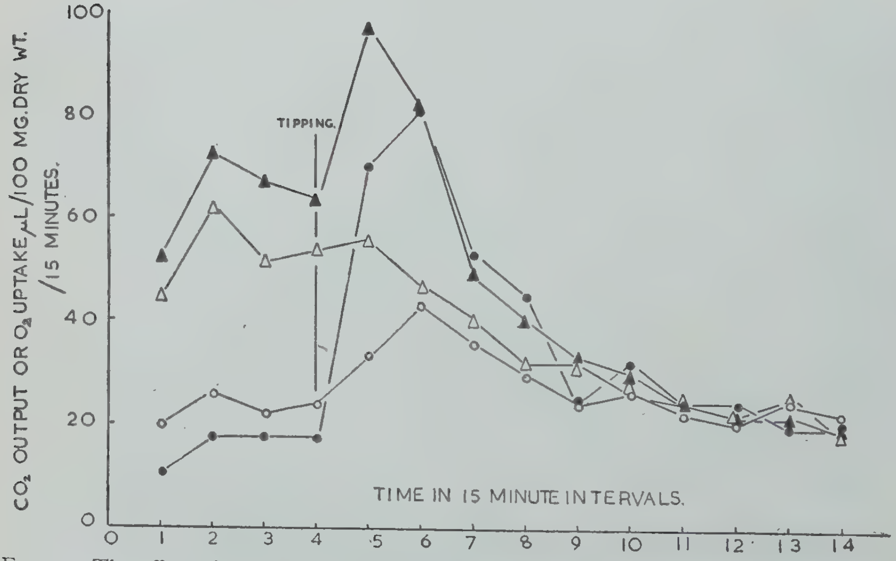

FIG. 14. The effect of 25 mM. NaF upon the respiration of discs in sucrose solution and in water.

–△– –▲– O_2 and CO_2 of samples in sugar.
–○– –●– O_2 and CO_2 of samples in water.

It is known from previous experiments that sugar uptake is almost abolished after application of fluoride. These results are extremely similar to the effects of dilute azide and D.N.P. upon beech mycorrhizas (Harley and McCready, 1953; and Harley *et al.*, 1956) which have been explained as due to uncoupling of phosphorylation from respiration and the release of aerobic fermentation. It will be important to carry this comparison further experimentally.

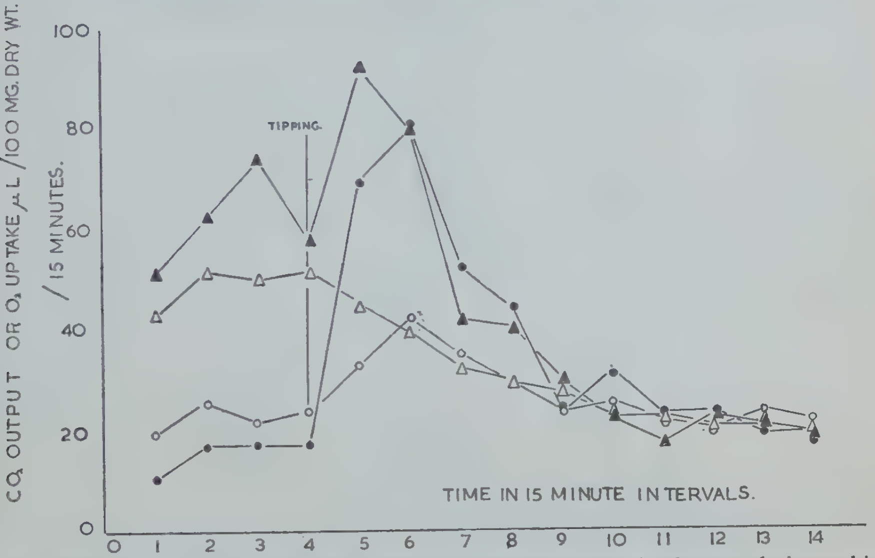

FIG. 15. The effect of 0·25 mM. NaF upon the respiration of discs in glucose solution and in water, symbols as in Fig. 14.

Sugar absorption by algal and fungal tissues

The technique of separation of symbiotic structures into their constituent tissues has proved useful in the study of ectrotropic mycorrhiza especially as an approach to the study of the functioning of the whole organs. The dissection technique outlined in the section on methods proved suitable to the same purpose with *Peltigera* discs. One previous attempt at such a study was carried out by Sosa-Bourdouil (1944) by separating the central chondrioid axis of *Usnea barbata* (Web) from the outer cortical and algal layers. The labour of the dissection of *Peltigera* imposed limitations on the number of replicates possible in any given experiment so that confirmation of results was sought in repetition of whole experiments.

The results of four experiments using 2 per cent. sucrose are presented in Table XIV. The most striking features are: the great difference between the sugar uptake rates by the algal and medullary layers giving ratios of uptake $\frac{\text{algal zone}}{\text{medulla}}$ of 3·1–6·8 with a mean of 3·9 and, the similarity of production of reducing sugar so that the ratio of rates $\frac{\text{algal zone}}{\text{medulla}}$ is 1·1.

The remaining results shown in Table XIV confirm the value of the

Table XIV

Behaviour of Dissected Discs, 'Dissectible' Quarter Discs, whole 'Dissectible' Discs, and 'Undissectible' Discs, on 2% Sucrose

N.B. All values for uptake, reducing sugars, and total sucrose utilized, expressed as mg. sucrose/30 discs/24 hours at 25° C. Final dry weights as mg./30 discs.

Experiment No. and date	11 (15/7/53)				14 (10/10/53)				17 (23/11/53)				18 (7/12/53)			
Medium, pH and buffer	Water (pH Ca 6·9)				M/100 Phthalate, pH 7·0–6·4				M/100 Phthalate, pH 5·6				M/100 Phthalate, pH 5·6			
	Uptake	Reducing sugars	Total sucrose utilized	Final dry weight	Uptake	Reducing sugars	Total sucrose utilized	Final dry weight	Uptake	Reducing sugars	Total sucrose utilized	Final dry weight	Uptake	Reducing sugars	Total sucrose utilized	Final dry weight
Algal zones	10·5	20·3	30·8	52·0	15·4	22·8	38·2	50·0	12·3	36·8	49·1	50·3	13·3	38·2	51·5	50·4
Medullary zones	2·3	13·4	15·8	51·7	5·0	31·6	36·6	51·5	1·8	30·4	32·2	37·3	4·1	37·2	41·3	44·9
Total of algal and medullary zones	12·8	33·7	46·6	103·7	20·4	54·4	74·8	101·5	14·1	67·2	81·3	87·6	17·4	75·4	92·8	95·3
'Dissectible' quarter discs	—	—	—	—	19·6	47·0	66·6	104·3	18·7	46·7	65·4	92·6	19·9	46·6	66·5	100·9
Whole 'dissectible' (i.e. thick) discs	15·1 17·2	29·3 24·4	44·4 41·6	94·2 103·9	15·2	34·2	49·4	99·0	17·9	36·6	54·5	91·9	—	—	—	—
Whole 'undissectible' (i.e. thin) discs	—	—	—	—	18·4 17·6	16·3 16·2	34·7 33·8	75·7 81·4	19·0	37·6	56·6	75·6	17·3 19·1	39·1 36·3	56·4 55·4	83·1 78·6

dissection technique by showing that the changes due to manipulation of selected material are not excessive. The slightly greater production of reducing sugar by dissected discs is expected from their greater surface area in contact with the sugar solution.

TABLE XV

Effect of M/50 NaF *on Dissected Discs on 2% Sucrose Media, pH 5·6 (M/100 Phthalate). The results of two experiments, No. 17 (23/11/53) and No. 18 (7/12/53)*

Uptake, reducing sugars, total sucrose utilized expressed as mg. sucrose/30 discs/24 hours at 25° C. Vol. of medium = 5 ml.

'Control' = sample without M/50 NaF in medium.

	Expt. No.	Uptake		Reducing sugars appearing in medium		Total sucrose utilized		Final oven dry weight (mg.)	
		Control	M/50 NaF	Control	M/50 NaF	Control	M/50 NaF	Control	M/50 NaF
Algal zones	17	12·3	0·1	36·8	37·7	49·1	37·8	50·3	34·1
	18	13·3	1·4	38·2	47·0	51·5	48·4	50·4	34·4
Medullary zones	17	1·8	0·9	30·4	27·0	32·2	27·9	37·3	31·9
	18	4·1	2·7	37·2	37·4	41·3	40·1	44·9	36·6
Total of algal and medullary zones	17	14·1	1·0	67·2	64·7	81·3	65·7	87·6	66·0
	18	17·4	4·1	75·4	84·4	92·8	88·5	95·3	71·0
'Dissectible' quarter discs	17	18·7	5·0	46·7	45·7	65·4	50·7	92·6	73·7
	18	19·9	4·2	46·6	48·8	66·5	53·0	100·9	78·4

TABLE XVI

Results of Experiment on the Effect of Addition of Sucrose to Medium on Respiration Rates of Dissected Discs. Expt. No. 32 (16/6/54)

Respiration rates (as μl./100 mg. dry weight/hour), R.Q. values, for before and after sucrose tipping, and ratios $\frac{CO_2 \text{ increase with sucrose}}{O_2 \text{ increase with sucrose}}$ values for 'undissectible' discs in the same experiment are given for comparison.

		Rates before sucrose tipped		Rates after sucrose tipped		Increase in rate with sucrose		Final dry weight (mg.)
		Rates	R.Q.	Rates	R.Q.	Increase in rate	$\frac{CO_2 \text{ increase}}{O_2 \text{ increase}}$	
Algal zones	CO_2 output	94	0·80	306	1·33	212	1·86	35·7
	O_2 uptake	117		231		114		32·9
Medullary zones	CO_2 output	66	0·74	149	1·24	83	2·68	32·2
	O_2 uptake	89		120		31		32·6
'Dissectible' quarter discs	CO_2 output	—	—	—	—	—	—	66·3
	O_2 uptake	—		207		—		70·2
Whole 'undissectible' discs	CO_2 output	103	0·89	311	1·30	208	1·71	53·9
	O_2 uptake	116		238		122		53·8

Table XV shows the effect of fluoride upon the two zones. The uptake of the algal zone is greatly reduced and that of the medullary zone less so. The change of reducing sugars in the media falls into line with the results on whole discs.

The results of measurements of O_2 absorption and CO_2 emission by separate algal and medullary zones are shown in Tables XVI and XVII. Sucrose

TABLE XVII

Results of Experiment on the Effect of Addition of Sucrose to Medium on Respiration Rates of Dissected Discs. Expt. No. 34 (24/6/54)

Respiration rates (as μl./100 mg. dry weight/hour), R.Q. values, for before and after sucrose tipping, and ratios $\frac{CO_2 \text{ increase with sucrose}}{O_2 \text{ increase with sucrose}}$ values for 'undissectible' discs in the same experiment are given for comparison.

		Rates before sucrose tipped		Rates after sucrose tipped		Increase in rate with sucrose		Final dry weight (mg.)
		Rates	R.Q.	Rates	R.Q.	Increase in rate	$\frac{CO_2 \text{ increase}}{O_2 \text{ increase}}$	
Algal zones	CO_2 output	74	0·76	299	1·34	225	1·79	36·0
	O_2 uptake	97		223		126		36·3
Medullary zones	CO_2 output	33	0·50	112	1·19	79	2·82	34·6
	O_2 uptake	66		94		28		35·8
'Dissectible' quarter discs	CO_2 output	55	0·73	238	1·36	183	1·89	71·7
	O_2 uptake	75		172		97		74·5
Whole 'un-dissectible' discs	CO_2 output	61	0·69	(253)	(1·21)	(192)	(1·60)	53·4
	O_2 uptake	89		(209)		(120)		57·0

(Figures in brackets = values from another sample in experiment)

TABLE XVIII

Uptake from 2% Sucrose Media, and Apparent Conversion of Sucrose to Reducing Sugars by Four Lichen Species in Comparison with Peltigera polydactyla

N.B. Initial sucrose content of each 5 ml. medium = 99·4 mg.

Species	Type of sample	Uptake (mg./sample/24 hrs.)	Reducing sugars appearing in medium (mg./sample/24 hrs.)	Total sucrose utilization (mg./sample/24 hrs.)	Final dry weight of samples	Mean uptake (mg.) per 100 mg. Final dry weight per 24 hrs.
Cladonia elongata	60 podetial tips	14·0	78·8	92·8	148·1	9·1
		13·3	80·0	93·3	151·8	
Cladonia cornuto-radiata	57 podetial tips	10·8	86·2	97·0	147·8	7·0
		10·2	86·1	96·3	153·1	
Parmelia physodes	25 thallus tips	17·5	79·5	96·0	143·5	12·8
		17·7	80·0	97·7	132·2	
Peltigera rufescens	27 discs (7 mm. diam.)	36·4	39·2	75·6	124·1	31·1
		39·4	32·6	72·0	120·1	
Peltigera polydactyla	27 discs (7 mm. diam.)	15·1	29·3	44·4	69·1	22·4
		17·4	24·4	42·8	76·2	

application in each case results in a respiratory rise with rise of R.Q. which is greater for the algal zone. This again indicated that respiratory rise is associated with absorption. The sugar uptake of the two zones in these experiments (Table XVI) indicates that the respiratory stimulation of the medullary zone was greater per mg. sugar absorbed than that of the algal zone.

Experiments with other lichen species

Certain experiments were carried out with other lichen species which serve to show two points. First, that suitable samples can be taken to allow reasonable replication of experimental results. Secondly, that the main points made about *Peltigera polydactyla* are widely applicable to the lichens.

Table XVIII shows the effect of samples of four species upon a 2 per cent. solution of sucrose. In each case a great release of reducing sugars into the media occurred together with a considerable uptake of sugar. The nature of the samples is described in column 2 of the Table and indicates the kind of material from which satisfactorily close agreement of replicates was obtained. Other experiments showed that the sensitivity of inversion by *Cladonia elongata* to pH was similar to that of *P. polydactyla* and that addition of phosphate to the media had no effect on sugar uptake.

DISCUSSION

The characteristic features of sugar uptake by the lichen thalli studied here are the greater rate of glucose uptake than fructose uptake from mixed solutions, and the absence of sucrose absorption. The evidence for the latter point may be marshalled as follows:

1. In sucrose solution *Peltigera* discs bring about a rapid release of reducing sugars.
2. The production of reducing sugars is a process which is independent of absorption since those factors, drying at 75° C., silver nitrate, sodium fluoride, and low pH, which inhibit or diminish absorption bring about an increased release of reducing sugars.
3. The uptake of sugar from a mixture of glucose and fructose in equivalent quantities is similar in rate to the uptake from sucrose.
4. The residual fructose after absorption from sucrose or an equimolar solution of glucose and fructose exceeds the residual glucose and is very similar in quantity in the two cases.
5. At high pH values or in 10^{-3} M. silver nitrate where reducing sugar production from sucrose is greatly diminished, uptake is greatly inhibited.
6. Similar curves of sugar uptake from sucrose solution are obtained when the uptake is plotted against total hexose concentration whether the latter be varied by pH or by initial sucrose concentration.

The process of absorption therefore appears to differ from that of tomato roots described by Dormer and Street (1949) and Street and Lowe (1950) where sugar absorption from sucrose exceeded absorption from glucose or fructose or equimolar mixtures of them. Again sugar absorption of *Peltigera* differs from tomato roots in that although having a sensitivity to pH, it has no well-defined optimum, but a high plateau between 5·0 and 8·0 for glucose and an ill-defined optimum around pH 5·5 for sucrose. Similarly, the evidence of Doudoroff (1951) for utilization of intact sucrose by *Pseudomonas* species suggests that the mechanism differs also in that case.

Brown (1952) suggests that in *Cucurbita* roots absorption of hexoses is independent of cleavage of sucrose. Many observers have recorded the greater release of fructose than glucose from sucrose (Rheinhold, 1949; Tobla and Ghanem, 1954; Dormer and Street, 1949; Street and Lowe, 1950) but a variety of mechanisms has been suggested and may indeed all be applicable to their specific cases.

The evidence that enzymes are at cell surfaces has recently been a subject of review by Rothstein (1954). This subject of fairly recent growth is undoubtedly of fundamental importance in studies of the movement of substances between the cells of multicellular organisms and also between host and parasite in pathological material and in symbiosis. There is little doubt that the enzymatic cleavage of sucrose by the lichens studied falls into this category, and evidence is already available that such an enzyme system is present in various fungi (yeast, Thorsell and Myrbach, 1951; *Myrothecium verrucaria* and *Aspergillus luchuensis*, Mandels, 1951, 1953; Beech mycorrhiza, Jennings unpublished; and *Neocosmospora vasinfecta*, Harley and Smith unpublished).

The difficulties of sampling lichen material for physiological work are superable when approached in the manner described in this paper.

SUMMARY

1. A technique of sampling *Peltigera polydactyla* for physiological work is described, together with estimates of variability.

2. The technique is applied to a study of sugar absorption.

3. Factors affecting sugar absorption were studied and the process shown to be dependent on metabolic activity.

4. A sucrose-splitting enzyme system separate from the sugar-absorption mechanism is shown to be held on the cell surface and to be equally active in the algal and medullary zones of *Peltigera*.

5. The main site of uptake of sugars is the algal zone. The fungal medulla is less active in uptake.

6. Preliminary experiments on other lichen species show similar features to *Peltigera*.

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Colchicine Effect on the Prothalli of *Goniopteris multilineata* (Wall.) Bedd. and *G. prolifera* (Roxb.) with Emphasis on Abnormal Spermatogenesis in Polyploid Prothalli

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With Plates XXV and XXVI and nine Figures in the Text

ABSTRACT

Cytological investigation into abnormal sperm formation in polyploid prothalli reported earlier has been undertaken. It is established that the B-type sperms are diploid and giant C type derive from tetraploids. The spermatogenesis in tetraploid prothalli is modified by frequent amitosis and fragmentation of the mother cell nucleus resulting in twins, triplets, and monsters with double or triple heads or tails; obviously the nuclear constitution of these sperms is variable.

INTRODUCTION

IN a recent paper the senior author (1952) reported the effect of dilute colchicine solutions on the gametophytes of two species of ferns, namely *G. prolifera* and *Dryopteris subpubescens*. The abnormal polyploid prothalli were found to produce abnormal sperms. Both the species in the case of probable diploid prothalli produced large B-type sperms while the probable tetraploid ones produced giant C-type sperms. Besides these giant C-type sperms, monsters were produced which were double headed or in which two, three, or four bodies were all united in front. No cytological study was undertaken then.

The present paper deals primarily with cytological studies in the polyploid prothalli. The various types of abnormal sperms reported earlier have again been observed to be produced by colchicine-treated prothalli not only of the two original species but also of a number of other ferns like *G. multilineata* (Wall.), Bedd., *Athyrium schimperi* Moug. ex Fee, *Diplazium esculentum* (Retz.) Sw., and *Pteris longifolia* L. The features of reaction being essentially similar, the results in the cytology of spermatogenesis responsible for the production of abnormal sperms are considered only for the two species *G. multilineata* and *G. prolifera*.

The genus *Goniopteris* belongs to the family Aspidiaceae (Copeland, 1947) while Holttum (1949) includes it in Thelypteridaceae. *G. multilineata* is an inhabitant of ravines and thrives best near waterfalls. The ripe spores of this species were collected from Mussoorie (W. Himalayas) in the month of August. *G. prolifera* grows along water channels at Amritsar (Panjab) in

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rather shady and humid localities. The plant assumes a straggling habit and buds are present in the axils of pinnae. The ripe spores in both the species are dark brown in colour with a well-developed perispore. The exine is loosely reticulately sculptured.

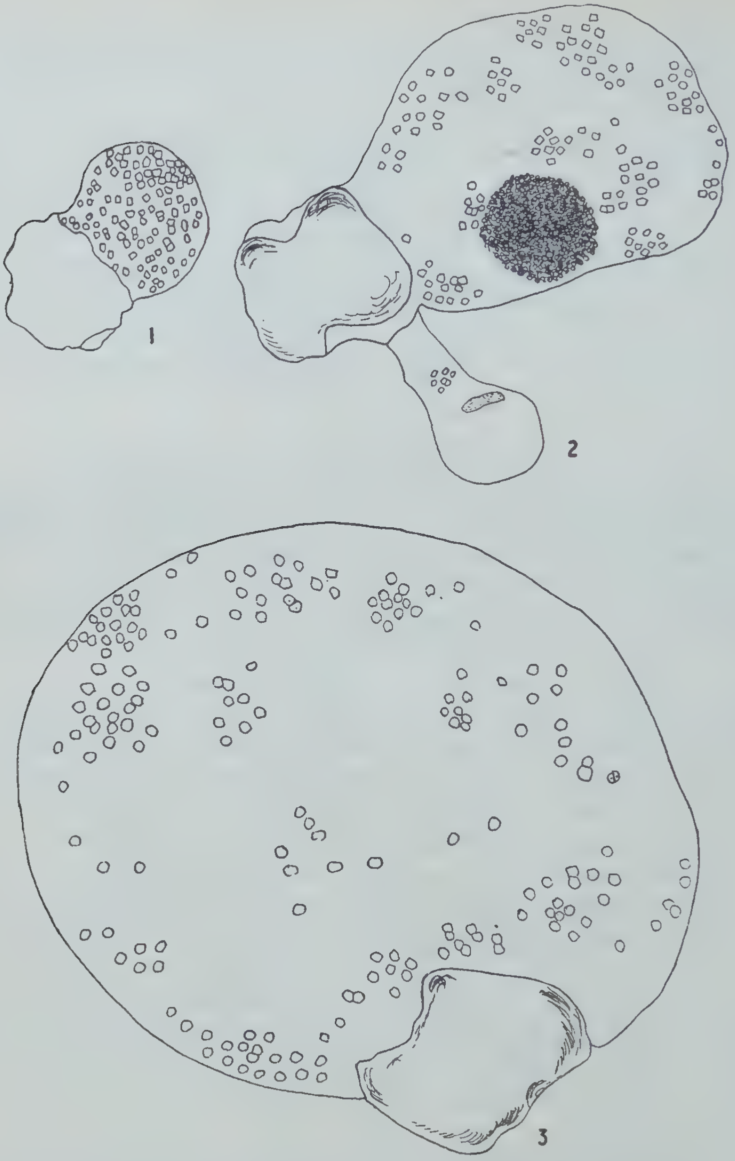
The method for germination of spores, &c., was the same as that followed earlier (Mehra, loc. cit.). The colchicine strength employed was chiefly 0·5 per cent. For the purpose of cytological studies the prothalli were fixed in acetic-alcohol at various stages of development and acetocarmine squash methods employed. A similar technique was followed for the study of spermatogenesis. The motile sperms from the normal and polyploid prothalli were fixed over osmic acid fumes and later stained with 1 per cent. acid-fuchsin, slides being passed through various grades of alcohol and xylol to the final mount in balsam.

OBSERVATIONS

The meiotic chromosomes in two species are shown in Figs. 1 and 2, Pl. XXV. It is obvious that both the species have $n = 36$. Incidentally it may be mentioned that Manton and Sledge (1954) report a chromosome number in *Goniopteris prolifera* as $2n = ca\ 72$ from Ceylon. The meiosis is perfectly normal and consequently the spores produced are genetically balanced and give rise to normal haploid prothalli with 36 chromosomes.

A microscopic study of colchicine-treated cultures of both the species, as well as of the other species mentioned above, showed that the response to the drug follows a uniform plan. The first papillate cell and filamentous protonema formed therefrom differed markedly from those in the control cultures both qualitatively and quantitatively (Text-figs. 1–3). For instance the first papillate cell became balloon-shaped, showing the characteristic 'nucleo-chloroplast sphere' reported previously. Similarly the rhizoids also showed response to the drug. In certain cases the rhizoids showed dichotomous or regularly pinnate branching while remaining all the time uninucleate and unicellular (Fig. 3, Pl. XXV). The number and orientation of chloroplasts in the balloons differed markedly from those in the control culture. They were relatively few as compared to the volume of the cell and it appeared as if their multiplication did not keep pace with the enlargement of the cell. The distribution was also lax with a tendency to be more peripherally located than in the centre (Text-fig. 3).

After a lapse of 30 days the drug-treated cultures were transferred to pure Knop's solution (half strength) for further growth. A week after removal from colchicine treatment the sporelings recovered and grew rapidly. The cultures at this stage were carefully scrutinized and it was noticed that in the same culture the filaments varied greatly in cell and rhizoid sizes. Some of them were abnormally large and more sturdy and deep green than the others. In all, three types of filaments were recognized. The smallest were haploid in constitution, with 36 chromosomes and comparable in all respects with the filaments in the control cultures. These obviously had not been affected by



FIGS. 1–3. *G. multilineata.* Normal, diploid, and tetraploid sporelings respectively. Nucleo-chloroplast sphere not shown in Fig. 3. (All × 500.)

the drug and later produced normal sperms. There were others intermediate in size which later produced exclusively the large B-type sperms. The third type had gigantic cells which produced some giant C-type sperms but mostly double-headed, double-tailed, and other monstrous types like twins, triplets, &c.

Two squashes from cells of the intermediate type are shown at metaphase in Text-figs. 4 and 5 in which 72 chromosomes can be counted with precision. Such gametophytes are hence diploid and so are the B-type sperms produced by these prothalli. The situation in the 'gigantic' filaments is, however, not so easy to determine. A number of squashes were made in which up to 140 chromosomes could be counted with a fair degree of accuracy (Text-fig. 6). It is probable that due to the large number of chromosomes a few may have been missed but one need not also expect an exact multiple as during colchicine doubling not infrequently a few chromosomes scatter out and are not included in the restitution nuclei (Mehra, 1946). In any case it seems certain that the giant C-type sperms produced from such prothalli are tetraploids or near that level. A queer filament belonging to this class was observed in which the terminal cell had bifurcated to form a cross-legged structure (Text-fig. 7).

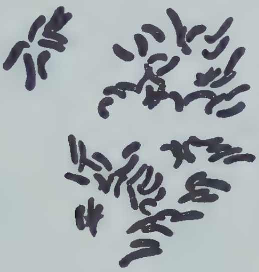


FIG. 4. *G. prolifera.* Somatic metaphase in a diploid filament $2n = 72$. (× 1150.)

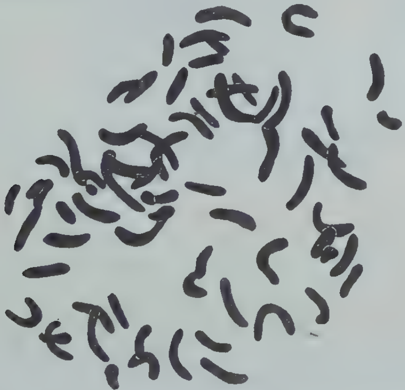


FIG. 5. *G. multilineata.* Metaphase in an antheridial initial of a diploid gametophyte $2n = 72$. (× 1760.)

All the cultures were transferred to soil after about 20 days. The fully developed diploid prothalli during their further growth assume a cordate appearance but tetraploids never do so and almost invariably remain ameristic, thus confirming previous observations. Interestingly enough some of them become highly branched and assume a 'trichomanoid' habit (Text-fig. 8). Almost every rhizoid borne on these is irregular and branched and sometimes of a

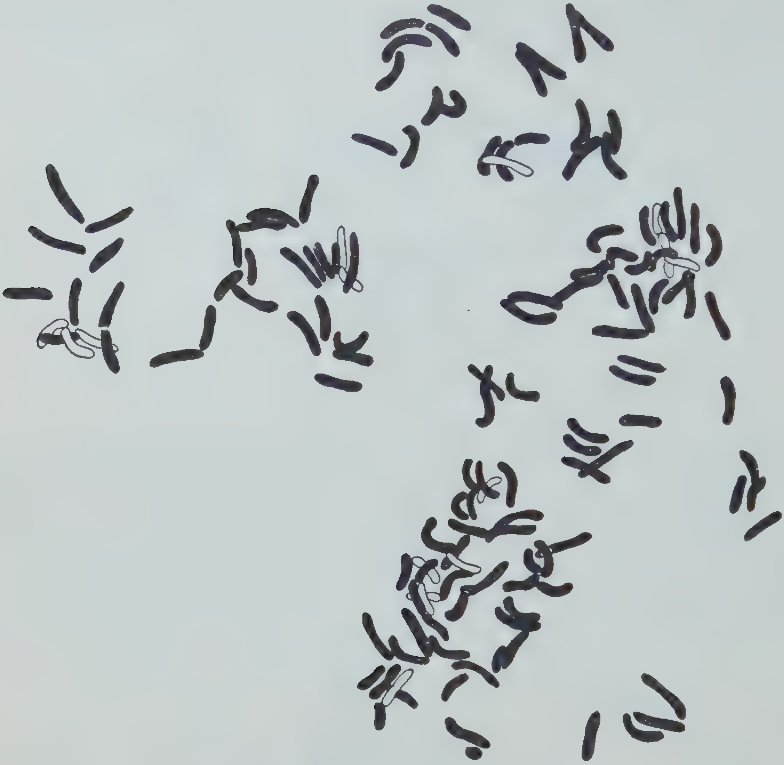


FIG. 6. *G. prolifera.* Metaphase in a cell of a tetraploid filament $4n = ca140$. (×1760.)

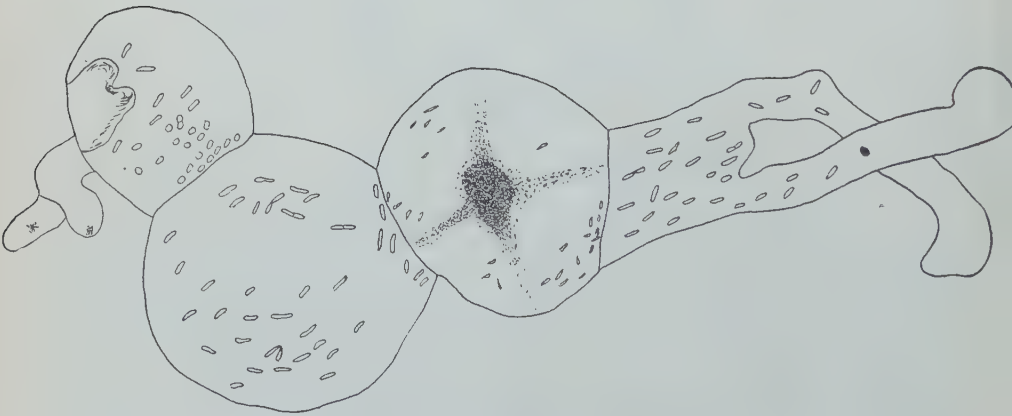


FIG. 7. *G. multilineata.* A tetraploid filament with the apical cell bifurcated. (×200.)

branched-tufted type without any cross septa, with internal continuity of branches and single nucleate. It is well known that unicellular glands are present on the prothalli of *Goniopteris*. The glands on these tetraploid prothalli were massive and often bifurcate, tuning-fork like or in other cases trifurcate (Text-fig. 9). The nucleus in these may lie either in the region of bifurcation or it may be confined to one lobe only. These prothalli are observed to produce antheridia only and rarely archegonia. The development of the antheridium in the case of all the species conforms to the typical leptosporangiate type but the cap cell shows a nipple-like bulging which is markedly prominent, and glistens when the living culture is seen under a binocular microscope.

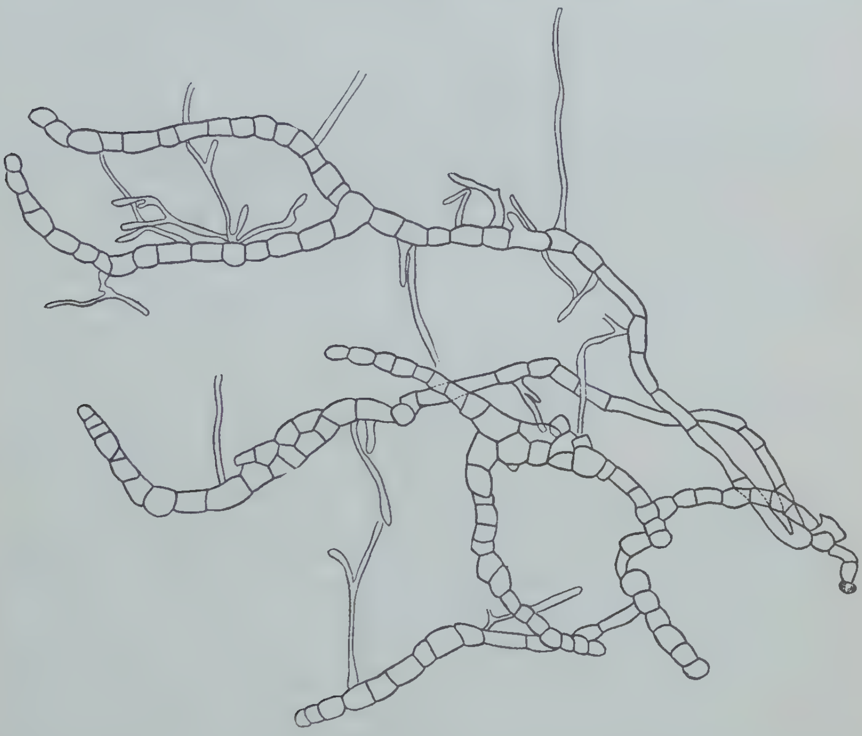


FIG. 8. *G. prolifera.* A 'trichomanoid' tetraploid gametophyte. (× 33.)

The formation of spermatogenous tissue in the diploid gametophytes is normal and by equational division up to thirty-two sperm mother cells are organized. Each diploid spermatocyte nucleus undergoes normal spermatogenesis resulting in the formation of normal diploid sperms of B-type. Such diploid sperms when liberated in water were rather sluggish in movement as compared to the small normal sperms which possess darting movements (Mehra, 1952).

In the case of tetraploid gametophytes, in so far as the spermatogenous tissue is concerned it is formed by regular mitotic divisions. The divisions, however, do not proceed farther than the eight- or sixteen-celled stage. Therefore, the number of spermatocytes in this case is either half or one-fourth of those formed in the diploid prothalli. Besides these spermatocytes, a few small-sized spermatocytes may also be formed by a process of amitosis which sets in during the process of sperm organogenesis. This will be discussed more fully in the later part of this paper.

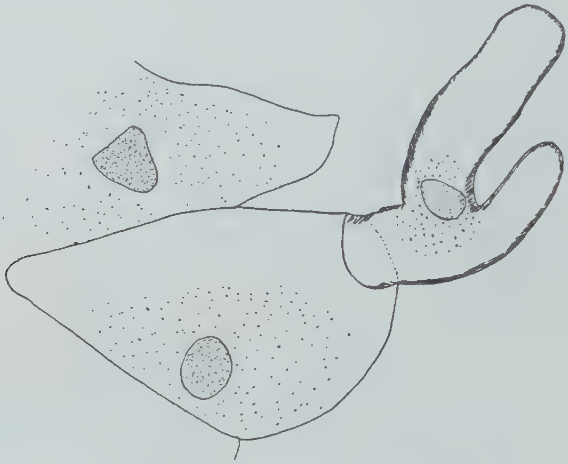


FIG. 9. *G. multilineata.* Bifurcated unicellular gland. (× 500.)

The spermatogenesis in these gametophytes is rather interesting and as a result abnormal sperms of various types are formed. It has been studied comprehensively in *G. multilineata* but similar results are noticeable in the other species also. Although in some cases the entire mass of the tetraploid nucleus transforms itself into a regular tetraploid sperm of giant C-type, yet in a great majority of cases it enters into what resembles the early prophase condition, as if in an attempt to have the reduced chromosome number. Since the pre-requisite conditions for reduction division are absent, recourse is taken to amitosis. Fig. 4 (bottom), Pl. XXV shows one such sperm mother-cell entering into this condition forming a dumb-bell-shaped structure. The prophasic condition is obvious. Quite often, this amitosis is complete and two nuclei of almost equal size are organized each of which becomes transformed into a sperm. The same figure (top) shows two such sperms in the process of formation. Obviously these sperms will be free from one another but it is difficult to adjudge their constitution. In other cases the amitosis may not be complete and when such a lobed nucleus enters into sperm formation, either a double-headed or a double-tailed sperm results. Fig. 5, Pl. XXVI shows a double-headed sperm in the process of formation. If amitosis is more or less complete but individual nuclei are not entirely separated, then the sperm organogenesis in such a mother cell results in a jointed twin (Fig. 6, Pl. XXVI). The sperms so formed may again be genetically unbalanced. In still other cases the cleavage of chromatic material may not only be highly unequal but also accompanied by the intrusion of cell wall (Fig. 7, Pl. XXVI). If the intrusion of the cell wall is completed, a small-sized spermatocyte having uncertain constitution of lower level results, as stated earlier. Such nuclei if transformed into sperms will be very small. In another spermatocyte (Fig. 8, Pl. XXVI) the nucleus has become trilobed and if the cleavage is completed it will give rise to a triplet, the like of which is shown in Fig. 9, Pl. XXVI. But if lobing remains shallow and nucleus transforms into a sperm, it is not expected to have a regular shape. A highest type of inequality in the size of these fragmented nuclei is shown in Fig. 10, Pl. XXVI, where a tetraploid spermatocyte nucleus has divided into three parts. The largest of these is in the process of sperm formation, having developed a good sized loop; the second one is still round while the third one which is by far the smallest does not yet give any indication towards sperm-formation.

CONCLUSIONS

The effect of the drug in disturbing the mechanism of cell division has been found to be variable as it does not affect all the germ cells of sporelings in a culture and consequently there are prothalli which do not show any response to the drug, others which are diploid and yet others tetraploid.

The physiological and genetic balance of the nucleus in diploid gametophytes is maintained and as a result the entire nucleus forms regular diploid sperms in their antheridia.

The behaviour of the tetraploid nuclei while undergoing spermatogenesis differs from those of the diploid. While some of the nuclei do form normal-looking giant sperms, the greater preponderance is of irregular types which are formed as a result of amitosis or cleavage of the nuclei. Thus in such cases the product of the same antheridium will consist of all types of abnormal sperms. It is not possible at this stage to explain adequately this uneven behaviour of the nuclei in the antheridia of these prothalli. However, the fact remains that there is a tendency exhibited by the tetraploid nuclei to revert to a condition with lesser number of chromosomes. In species with high chromosome number like *Dryopteris subpubescens* ($n = 72$) and *Pteris longifolia* ($n = 58$) there is a still greater preponderance of abnormal sperms.

All these types of sperms irrespective of their size and nuclear constitution are motile. Their movement is, however, variable. In the case of diploid and tetraploid sperms it is sluggish and in the case of highly abnormal ones more or less a state of immobility is exhibited, the sperm almost revolving round its axis. It would be of considerable interest to study the behaviour of these abnormal sperms in the act of fertilization. A study of the fate of the blepharoplast in such abnormal sperm formation is yet another point of interest awaiting investigation.

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Our thanks are due to Mr. R. S. Pathania for taking the microphotographs illustrating this paper.

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EXPLANATION OF PLATES

Illustrating P. N. Mehra and D. S. Loyal's paper on the effects of colchicine on the cytology of fern prothalli.

PLATE XXV

FIG. 1. Meiosis in a permanent aceto-carmine mount of *Goniopteris multineata* showing $n = 36$. ×2,340.

FIG. 2. Meiosis in *G. prolifera* showing $n = 36$. ×1,560.

FIG. 3. *G. prolifera.* The rhizoids showing dichotomous or pinnate branching in the drug-treated culture.

FIG. 4. *G. multilineata.* A tetraploid sperm mother cell (bottom) showing dumb-bell-shaped nucleus. In another mother cell two sperms are in the process of formation.

PLATE XXVI

FIG. 5. A double-headed sperm in the process of formation.

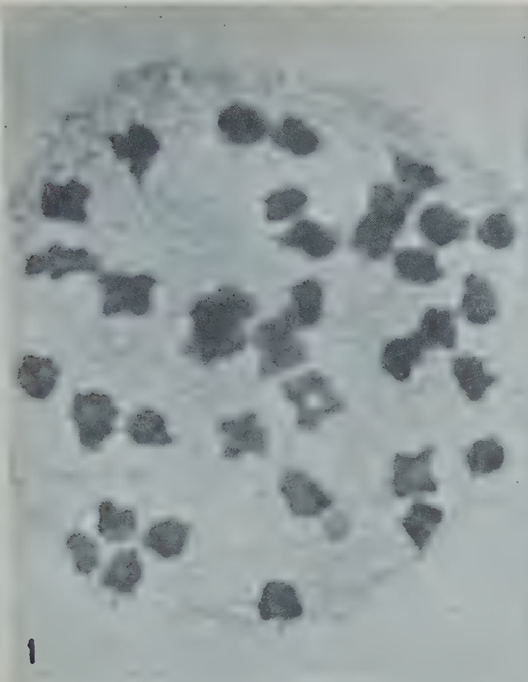
FIG. 6. Twin sperms which are somewhat unequal in size formed as a result of amitosis.

FIG. 7. The two highly unequal separate nuclei with invagination of the cell wall in a mother cell.

FIG. 8. The tetraploid nucleus has become trilobed.

FIG. 9. A triplet.

FIG. 10. Tetraploid nucleus divided into three parts amitotically, two of which are at different stages of sperm formation.



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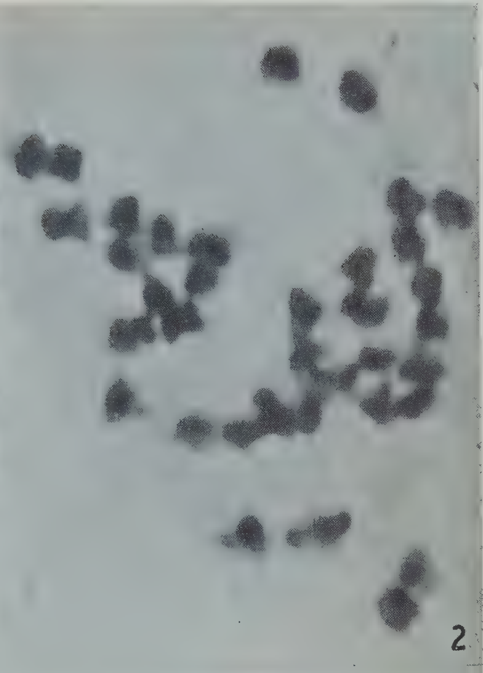

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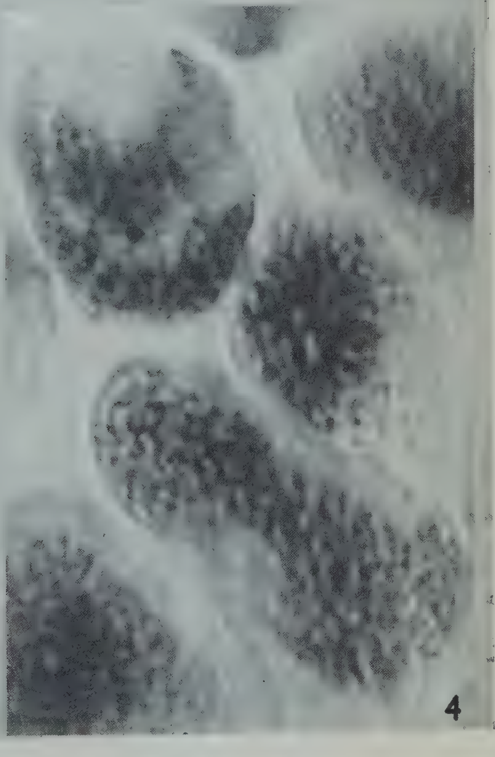

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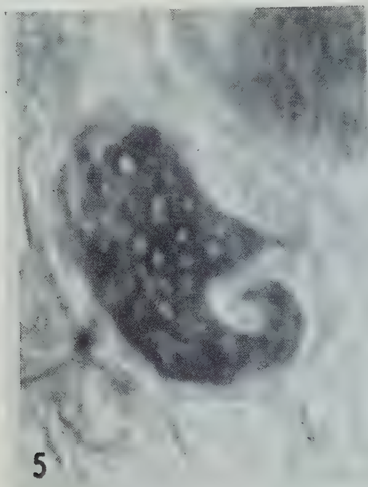
P. N. MEHRA *and* D. S. LOYAL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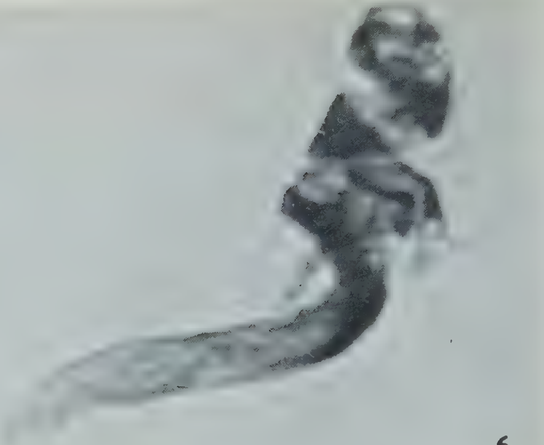
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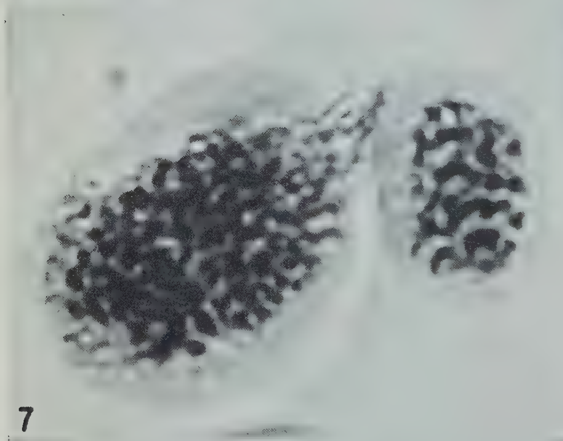
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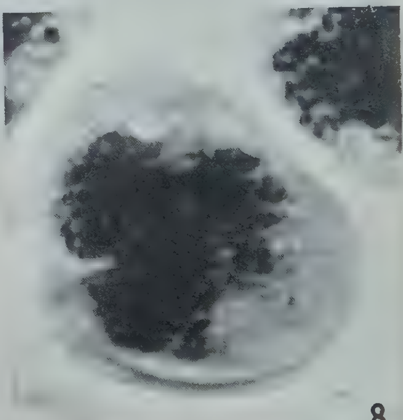
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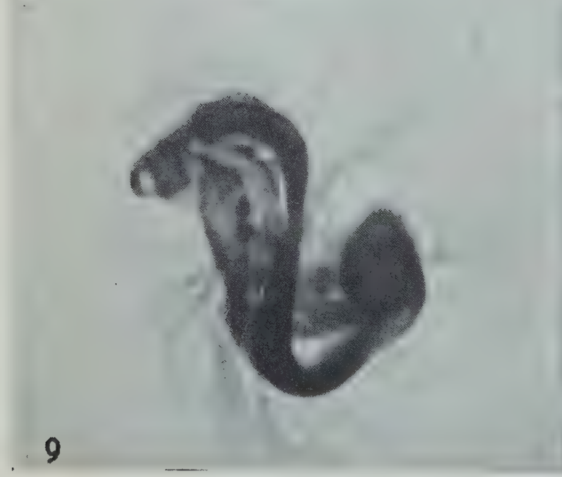
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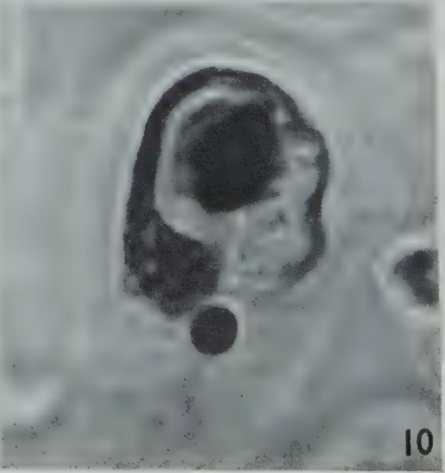
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P. N. MEHRA *and* D. S. LOYAL

Comparative Physiology of Apple Rootstocks

II. The Effect of pH and Aeration on the Growth of Malling Apple Rootstocks in Water Cultures

BY

I. F. BIRD AND H. C. RUCK

(Research Institute of Plant Physiology, Imperial College, London)

With two Figures in the Text

ABSTRACT

Three Malling apple rootstocks, M.IX, M.II, and M.XVI, were grown in aerated and non-aerated nutrient solutions at pH 3·6, 4·0, and 5·5, and their growth and survival compared with rootstocks grown in sand and in soil. No plants survived in the non-aerated cultures due to severe bacterial and fungal attack on the roots. In the aerated series a marked difference in susceptibility of rootstocks to root infection was found, M.XVI being considerably more resistant than M.II or M.IX. More rootstocks survived at the lower pH's (4·0 and 3·6) but among surviving plants of M.XVI growth was much less than at pH 5·5. It would appear therefore that factors making for survival do not of necessity make for optimum growth. In sand and soil cultures all plants survived.

MANY annuals and herbaceous perennials have been grown successfully in water cultures but woody plants grown for long periods of time have on the whole proved less successful. One of the difficulties encountered has been the death of the roots; thus with citrus, Chapman, Brown, and Rayner (1947) and Chapman (personal communication, 1954) found that the roots were very susceptible to fungal attack in water cultures at pH 5·5, but that the attack was controlled by lowering the pH to 4·0. Similar results have been reported with forest trees, e.g. conifers (Kramer, 1954, personal communication). Many efforts have been made in the past to grow apples in water cultures and difficulties have always been encountered. Barker (1920), Harris (1929) and Pearse (1937, 1939) have noted the susceptibility of apple roots to fungal and bacterial attack, and stressed the need for aeration of the culture solution.

In 1954 therefore experiments were carried out with three apple rootstocks at three levels of pH, grown in aerated and non-aerated nutrient solutions. The results of these experiments are presented here.

EXPERIMENTAL

Materials and Methods

Malling IX, a dwarfing rootstock, Malling II, an intermediate, and Malling XVI, a vigorous rootstock, were grown at pH 3·6, 4·0, and 5·5. The

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following complete nutrient solution made up with filtered tap water was employed.

Nutrient solution

Element	p.p.m.	Salt	gr./litre
K	45	KH_2PO_4	0·16
	45	K_2SO_4	0·10
P	35	KH_2PO_4	0·16
N	70	$Ca(NO_3)_2 4H_2O$	0·59
Ca	100	,,	0·59
	80	Tap water	
Mg	55	$MgSO_4.7H_2O$	0·57
N	56	$(NH_4)_2SO_4$	0·26

Iron was supplied as the ethylene diamine tetra-acetate complex (5 p.p.m. Fe). Trace elements were supplied as follows:

Zinc as zinc sulphate	0·06 p.p.m.
Copper as copper sulphate	0·06 ,,
Boron as boric acid	0·5 ,,
Manganese as manganese sulphate . . .	0·25 ,,

The pH of the solutions was maintained within ±0·2 by the addition of 5N H_2SO_4 or 5N NaOH as required. With the large volume of solution used very little adjustment was necessary.

The plants were grown in 30-gallon galvanized iron tanks which had been coated internally with three coats of Mullard's asphalt paint. Twelve such tanks were used: viz. aerated and non-aerated sets at each of the three pH levels, the whole series in duplicate. In the aerated solutions, air was forced at the rate of approximately 3 litres per minute through pinholes in a loop of small-bore polythene tubing which passed completely around the bottom of the tank. In this way a good distribution of fine air-bubbles was ensured. Twelve plants of each rootstock were grown in each tank, making 24 replicates per treatment.

A similar series of rootstocks was grown in 10-inch pots (*a*) in sand, supplied with the same nutrient solution at pH 5·0, (*b*) in John Innes potting compost (pH 6·5) which was top dressed with dried blood at fortnightly intervals. There were 6 replicates of each rootstock in both treatments. In this manner the growth of the rootstocks could be compared under different cultural conditions.

Routine observations

At the beginning of the experiment (30 March 1954) individual rootstocks were selected by weight for uniformity and planted. A representative sample of 12 plants of each rootstock was taken at this time for determination of dry weight, and the regression of dry weight on fresh weight was used for estimating the initial value for each plant. The final dry weights of the surviving plants were determined at the end of the experiment (15 September 1954), when the plants were divided into leaves, stems, and roots, dried at 90° C. and weighed. During the experimental period the oxygen concentrations of the solutions were determined at intervals by a modified Winkler method.

Survival of stocks

The plants were examined at weekly intervals and the condition of the root systems noted. As was anticipated, fungal (chiefly *Pythium* spp.) and bacterial infection occurred in the water cultures. After 6 weeks' growth the number of roots was counted and their lengths and the lengths of infected parts measured. There was no significant effect either of aeration or pH on the mean number or length of the roots at this time, although the roots of M.II were significantly less both in number and length than those of the other two stocks ($P = 0{\cdot}05$) (Table I).

TABLE I

Mean Root Number and Mean Root Length (cm.) after Six Weeks' Growth

	M.XVI	M.IX	M.II
Root No.	7·7	7·1	5·8
Root length (cm.) . . .	28·7	29·5	21·6

Root infection

An analysis of variance was carried out on the severity of root infection, which was measured as length of infected root expressed as a percentage of whole root. The analysis showed that the effects of stock and pH ($P = 0{\cdot}001$) and their interaction ($P = 0{\cdot}01$) were significant. Neither aeration nor any interactions involving aeration approached significance. The mean values are given in Table II.

TABLE II

Length of Infected Root expressed as a percentage of Whole Root. Sample taken after Six Weeks

pH *Rootstock*	3·6	4·0	5·5	Mean
M.XVI . .	0·8	0·6	1·5	1·0
M.II . .	2·5	3·1	28·0	10·9
M.IX . .	3·4	4·8	24·7	11·2
Mean . .	2·2	2·8	18·1	—

Least significant difference between two values in the body of the table = 8·2
Least significant difference between two values in either margin = 4·7

It thus appears that both the main effects and the interaction can be explained in terms of the very much greater infection for rootstocks M.IX and M.II at pH 5·5, there being no significant differences between the other variables.

Owing to the difficulties of measuring the rapidly growing root systems, no further measurements of root growth and number were made.

During the season a number of plants died in all the water cultures. Dead plants were removed at weekly intervals, and the death-rate (R) for all the water-culture plants was calculated using the formula:

$$R = \frac{100(\log_e N_{t2} - \log_e N_{t1})}{t_2 - t_1}$$

where Nt_n = number of plants surviving at a time t_n. The mean death-rate together with its standard errors for each month is shown in Fig. 1. It will be observed that the death-rate increases significantly with time. There were marked differences between treatments, and the number of survivors at weekly intervals is shown in Fig. 2. Although at first aeration had no effect, by the end of the season it proved to be the chief factor in survival, and all plants in the non-aerated cultures died. There were no deaths in either sand or soil cultures.

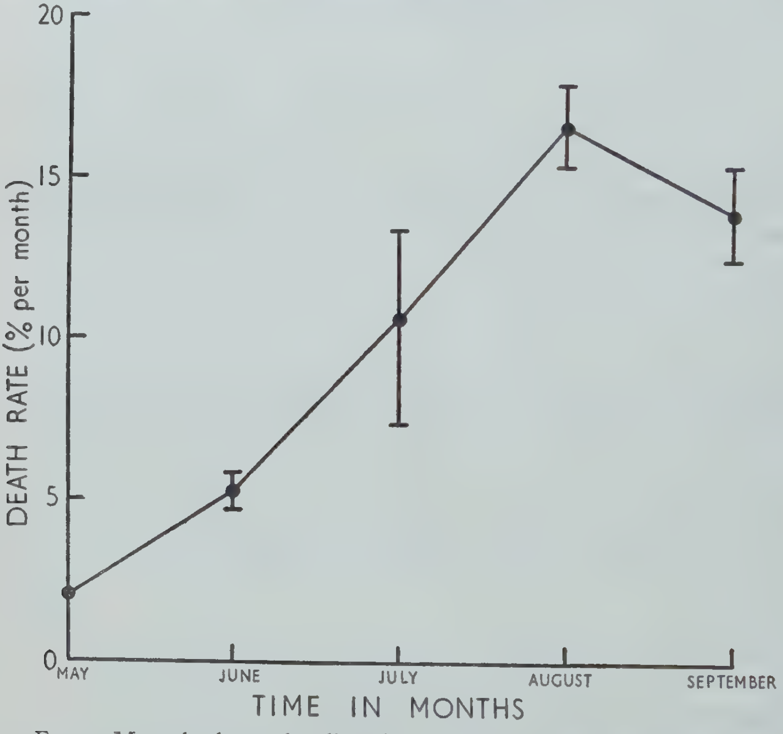

FIG. 1. Mean death-rate for all apple rootstocks in aerated water culture.

The initial and final dry weights of the plants are shown in Table III and the number of survivors in the aerated series in Table IV.

Although the largest percentage of M.XVI rootstocks survived at pH 4·0, growth as measured by increase in dry weight was almost twice as great at

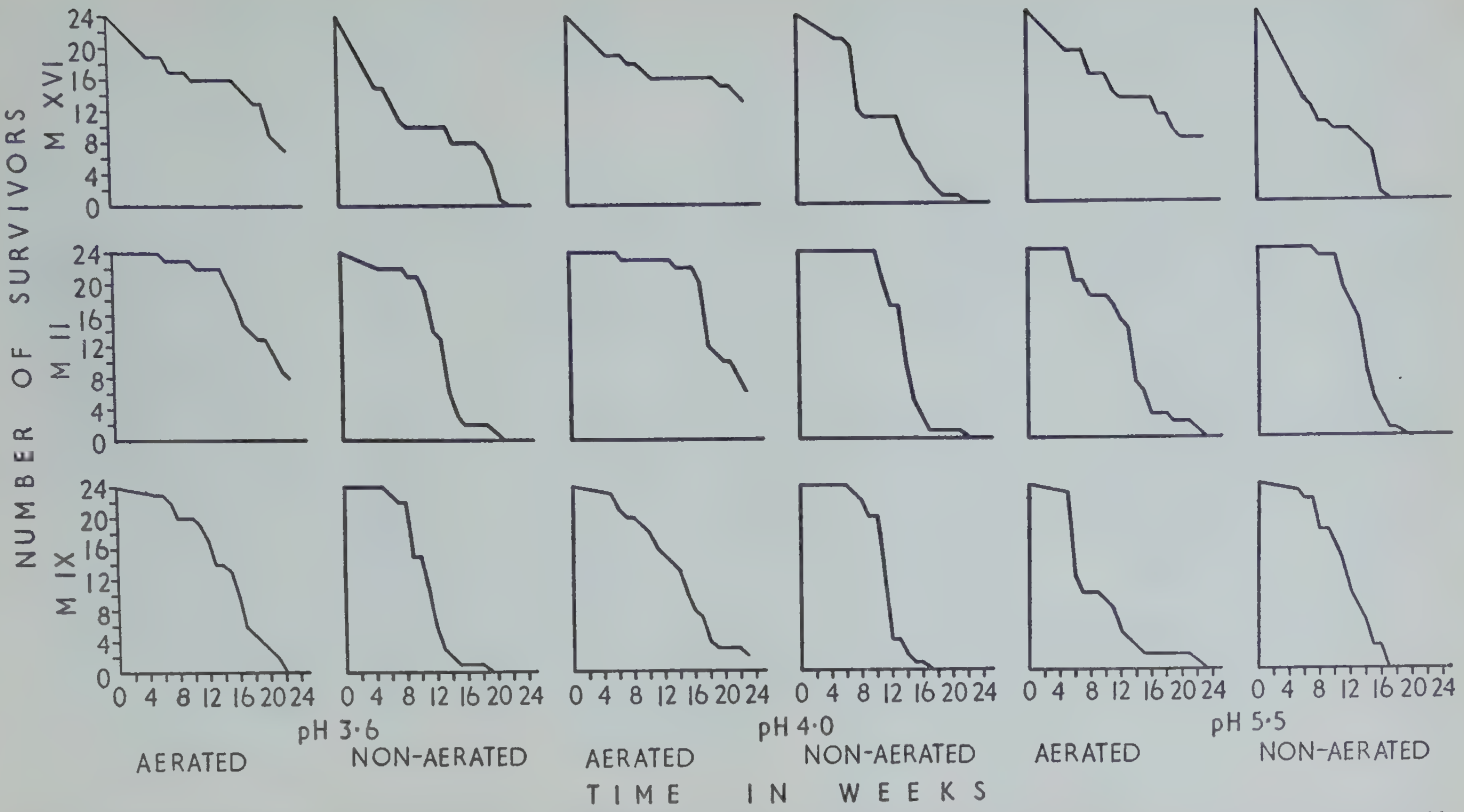

FIG. 2. Number of survivors out of a total of 24 for three rootstocks at various levels of pH, with and without aeration. Census of survival at 2-weekly intervals from setting up the cultures.

TABLE III

Initial and Final D.W. (g) of M.XVI, M.II, and M.IX Rootstocks under Different Cultural Conditions

Treatment	M.XVI		M.II		M.IX	
	Initial D.W. 30 March 1954	Final D.W. 15 Sept. 1954	Initial D.W. 30 March 1954	Final D.W. 15 Sept. 1954	Initial D.W. 30 March 1954	Final D.W. 15 Sept. 1954
Water cultures						
pH 3·6 aerated .	3·17±0·11	14·25±1·34	2·59±0·10	13·89±2·71	3·01±0·04	—
No. of replicates .	24	7	24	8	24	0
pH 4·0 aerated .	3·18±0·09	19·73±2·03	2·77±0·20	17·66±3·15	2·89±0·18	14·08±1·30
No. of replicates .	24	11	24	6	24	2
pH 5·5 aerated .	3·01±0·11	32·76±4·32	2·70±0·09	—	2·96±0·07	—
No. of replicates .	24	8	24	0	24	0
Soil culture . .	2·98±0·18	32·87±2·63	3·01±0·24	34·73±1·96	3·24±0·14	20·80±2·75
No. of replicates .	6	6	6	6	6	6
Sand culture . .	3·03±0·15	21·14±2·04	2·79±0·13	26·37±3·21	2·89±0·17	19·28±1·07
No. of replicates .	6	6	6	6	6	6

There were no survivors in non-aerated water cultures.

pH 5·5 as at pH 4·0, was better than in sand, and equal to that in soil cultures. It is of interest to note that although surviving M.XVI made best growth at pH 5·5, none of the M.IX or M.II rootstocks survived at this pH. At pH 3·6 growth and survival were somewhat less than at pH 4·0.

TABLE IV

Percentage Survival of Rootstocks at 15 Sept. 1954
Grown with Aeration

pH	3·6	4·0	5·5	Mean
M.XVI	29·2	45·8	33·3	36·1
M.II	33·3	25·0	0	19·4
M.IX	0	8·3	0	2·7
Mean	20·8	26·4	11·1	19·4

An analysis of variance of the data of survival at the final sampling in Table IV showed highly significant differences ($P = 0·001$) between the stocks and level of pH grown with aeration.

As there were no survivors in the non-aerated series it was impossible to test for interactions involving this treatment. A similar analysis of variance made on data obtained on 26 July, however, showed significant interactions (at the 0·05 level) between rootstocks, levels of pH, and aeration. Data are entered in Table V.

TABLE V

Interaction of Stock, Levels of pH, and Aeration, July 26, 1954

	Aerated			Non-aerated		
pH	3·6	4·0	5·5	3·6	4·0	5·5
M.XVI	54·9	55·5	47·4	35·1	27·1	12·5
M.II	60·4	73·8	19·2	16·7	19·2	19·2
M.IX	39·9	35·1	16·7	12·5	12·5	19·2

Least significant difference = 17·3

For purposes of statistical analysis the data were transformed to 'angles of equal information' and the values are presented in this form.

Oxygen determinations

The oxygen concentrations of the nutrient solutions were determined at the time of their preparation and at the end of each month just before the solutions were changed. An analysis was also carried out in the third month one week after changing. The results are shown in Table VI, and it will be seen that in the non-aerated solutions the level of oxygen had fallen to about the same value at the end of each month, and, at least in the last month, this was reached in the first week. In the aerated solutions there was little change in the oxygen concentrations throughout the period.

TABLE VI

Mean Oxygen Concentrations of Nutrient Solution

(c.c.s O_2 per litre N.T.P.)

	Aerated	Non-aerated
Freshly prepared solution	5·9+0·0 (n = 2)	
End of 1st month 13.5.54	5·6±0·05 (n = 6)	4·2±0·07 (n = 6)
Solution changed 14.5.54		
End of 2nd month 14.6.54	6·5±0·08 (n = 12)	3·5±0·06 (n = 12)
Solution changed 15.6.54		
Solution after one week 22.6.54	6·2±0·05 (n = 6)	3·5±0·20 (n = 6)
End of 3rd month 13.7.54	5·8±0·09 (n = 12)	3·8±0·20 (n = 12)

DISCUSSION

Aeration of the nutrient solution has been shown to be beneficial for the growth of most plants in water cultures, and the apple is apparently no exception. Thus Boynton and Compton (1943) found over a 2-month period that there was an almost linear increase in number and weight of apple roots with increased oxygen concentration over a range of 5 to 20·8 per cent. in the mixture of gases used for aeration. The present work has shown the importance of aeration, as by the end of the season there were no survivors in the non-aerated cultures—although the detrimental effect of the absence of aeration did not become significant for a considerable period (2 months). The figures for oxygen concentration of the non-aerated solutions show that within a week of changing the nutrient solution the oxygen content had fallen from 5·9 c.c.s. to 3·5 c.c.s per litre, and was still at this level after a further 3 weeks, i.e. until the solution was changed. It would therefore appear that the initial healthy growth in the non-aerated solutions followed by the increasing death-rate is not directly related to the oxygen content of the solutions but may be due to the development of pathogenic micro-organisms.

The factors making for survival remain obscure, but it is probable that the temperature of the culture solution is of importance. In this connexion Heinicke (1933) has shown that submergence of the roots of potted apple-trees during the growing season caused serious leaf injury particularly if there was hot weather during the period, and Kramer and Jackson (1954) found that with tobacco plants grown in flooded soils injury developed more rapidly in warm than cool soils.

Acidification of the soil or nutrient solution has been used by many workers as a means of combating root diseases of citrus, by Chapman and Brown (1942), and Chapman, Brown, and Rayner (1947), and of Poinsettia by Shanks and Keller (1954). The present experiments have shown that although more rootstocks survived at the more acid pH's, the total number of survivors was very small, 26·4 per cent. of all rootstocks at pH 4, and only 11·1 per cent. at pH 5·5 at the final sampling. Thus with apple rootstocks this means of control of disease is only moderately successful. Furthermore in spite of the death of all M.IX and M.II rootstocks at pH 5·5, the growth of those M.XVI rootstocks that did survive was approximately twice that made at pH 4·0. It would appear, therefore, that the factors making for survival do not of necessity make for optimum growth.

The results of the experiment demonstrate clearly that the rootstocks show greatly different degrees of resistance to root diseases, M.II and M.IX being extremely susceptible to attack. The poor growth of M.II in water cultures had been previously shown by Pearse (1937). Similarly in experiments carried out in Israel in the field, Barak (1954) has shown that rootstocks have very different susceptibilities to root diseases caused by *Rhizoctonia*, *Sclerotium*, and *Macrophoma*; M.II, M.IX, and Hashabbi being very susceptible and M.XVI almost immune. The factor determining infection is still unknown, and it is remarkable that no plants either in sand (pH 5·0) or soil (pH 6·5) were lost from this cause.

In view of further work it should be emphasized that throughout these experiments the stem of the cutting was immersed in the culture solution. In more recent experiments the stem was kept above the level of the solution and under these conditions the susceptible rootstocks M.II and M.IX have been satisfactorily grown in aerated water cultures.

SUMMARY

1. Three apple rootstocks, M.IX, M.II, and M.XVI, were grown at pH 3·6, 4·0, and 5·5 in aerated and non-aerated solutions. The susceptibility of the roots to fungal and bacterial attack and growth-rates of the rootstocks were determined and compared with rootstocks grown in sand and in soil cultures.

2. It was found that aeration had a beneficial effect on survival of the rootstocks, there being no plants alive in the non-aerated cultures at the end of the season owing to severe fungal and bacterial attack on the roots.

3. In the aerated solutions a marked difference in susceptibility to root disease was found, M.XVI being considerably more resistant to attack than M.II and M.IX.

4. The number of rootstocks surviving at the final sampling in the aerated series was greatest at the lower pH's (4·0 and 3·6), but growth of surviving M.XVI at pH 5·5 was approximately twice that at pH 4·0 and was as good as that of those grown in soil and better than those in sand. Relatively high pH was therefore not inimical to the growth of the plants provided they remained uninfected, and it would appear that the factors making for survival do not of necessity make for optimum growth. The factor determining infection is still obscure, though it is remarkable that no plants in sand or soil (pH 6·5) were lost from this cause. More recent experiments have demonstrated the importance of keeping the stem base out of the solution and when this has been done successful growth has been achieved.

ACKNOWLEDGEMENTS

Thanks are due to Professor F. G. Gregory and to Dr. B. D. Bolas for constant help and advice and to Dr. Pearce and Mr. Freeman of the Statistics Department of East Malling. We also acknowledge gratefully the kindness of Dr. F. R. Tubbs, director of East Malling Research Station, in providing facilities for this work to be carried out there.

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The Influence of Factors of the Aeration Complex and Light upon Fruit-body Form in Pure Cultures of an Agaric and a Polypore

BY

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(*Birkbeck College, London*)

With Plates XXVII–XXIX and two Figures in the Text

ABSTRACT

Methods involving controlled temperature and illumination, continuously renewed sterile culture atmosphere of defined gaseous composition and vapour pressure, and automatic replacement of water loss from the medium have been applied to the analysis of sporophore form in *Collybia velutipes* and *Polyporus brumalis*. In *P. brumalis* progressive suppression of the pileus but enhanced stipe elongation occurs as light intensity (over a certain range) or transpirational water-loss are reduced. These factors act additively. Cap expansion in *C. velutipes* requires light and is, in addition, inhibited by an atmospheric staling agent removable by KOH and replaceable by CO_2. Data and observations on conditions affecting times of production, numbers and dimensions of fruit-bodies are given, especially for *P. brumalis*. Other aeration factors are considered but shown to have no morphological effect.

INTRODUCTION

THE morphogenic mechanisms operating in a developing hymenomycete sporophore are not known, although hyphal behaviour underlying form changes has been described (Corner 1932, 1934, 1935, 1953) and theoretical consideration given to aspects of shape determination (Gurwitsch, 1921). That these mechanisms are sensitive to external factors is suggested by the large numbers of abnormal fruit-bodies reported from special situations such as caves, mines (Maheu, 1906, 1926; Spaulding, 1910) and the laboratory (Long and Harsch, 1918 and references in Bavendamm, 1936 and Lohwag, 1952). Such abnormalities, often despised by the taxonomist, are not valueless. On the contrary, if reproducible experimentally, they may be highly informative concerning mechanisms of normal development.

Most approaches to the subject have been qualitative or at best roughly quantitative and there is need of more precise definition of the external variables and more exact recording of the response of the fungus.

A long recognized formative requirement for many sporophores is light. Its effect on cap and stipe expansion in *Coprinus lagopus* has been the subject of important studies. Here heightened temperature (Borriss, 1934*b*) and mechanical stimulation (Borriss, 1934*b;* Stiefel, 1952) can to some extent substitute for light.

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Culture in small vessels early drew attention to the possible operation of atmospheric factors upon form. Thus the benefits of aeration, without analysis of its constituent factors, were shown by Lakon (1907), Schenck (1919), and Badcock (1943).

Undesirable abnormalities in cultivated mushrooms grown in ill-ventilated places have stimulated a search for specific atmospheric staling substances with morphogenic influence. Enrichment of small volumes of still air surrounding developing mushrooms with 1·8 per cent. of CO_2 caused restricted cap expansion and elongation of stipes (Lambert, 1933). The effect, which was not due to O_2 deficiency, gave way to irreversible stunting of growth at higher CO_2 tensions. Mader (1945) washed the air of poorly aerated mushroom houses. The washing agents effective in preventing abnormalities suggested that unsaturated hydrocarbons might be responsible for at least a component of the effect. Fruit-body response was poorly defined, but probably included a reaction to CO_2 of the kind described by Lambert (Mader, 1945, p. 1141, Expt. B.). The effect was not due to atmospheric humidity. Finally Stoller (1952) has shown that long stipes and reduced caps may occur in the absence of CO_2 and speculates on the nature of the volatile causative agents.

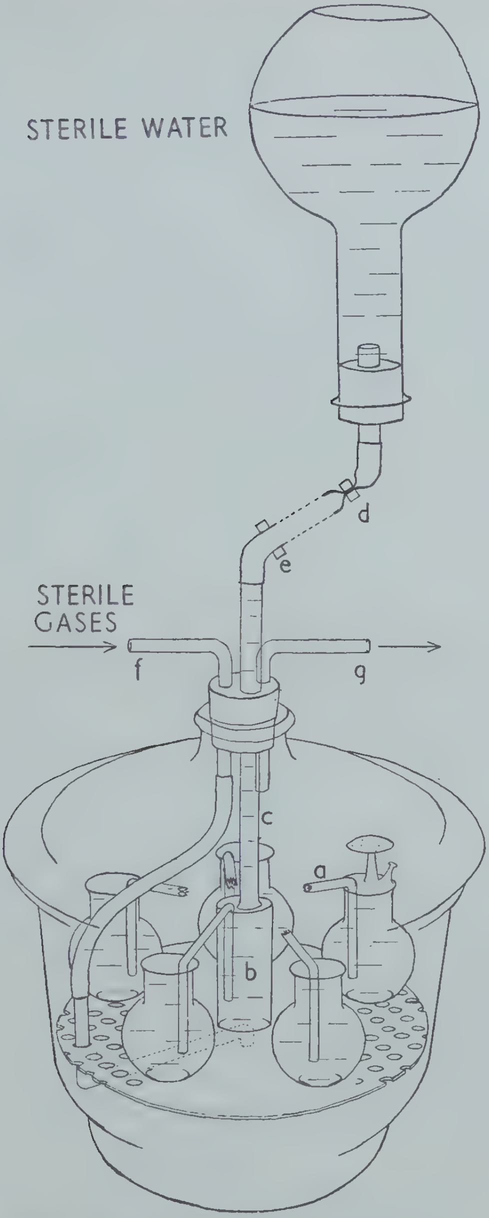
A highly distinctive mushroom abnormality is Rose-comb 'disease', characterized by brain-like outgrowths, often bearing hymenium, on the upper surface of the cap. Whether this is caused by an upsetting of the usual mechanisms controlling dorsoventrality of the cap, or is a hypertrophy of a tumour-like kind is not known. It appears to be correlated with the presence of mineral oil fumes (Lambert, 1930; Dillon-Weston and Taylor, 1942). Similar freaks were induced experimentally by aeration with unwashed laboratory air in *Coprinus ephemerus* (Keyworth, 1942) and are known in nature (Ulbrich, 1926; Legué, 1909).

Several workers have interpreted the greater normality of sporophores when aerated as due to greater transpiration rates (e.g. Lakon, 1907; Falck, 1913; Schenck, 1919) but clear if preliminary evidence of a transpiration effect appears first in Hopp's (1938) experiments on poplar-block cultures of *Ganoderma applanatum* fructifying in continuously renewed air. Neither very high nor very low saturation deficits were optimal.

Borriss (1934*b*), using still air conditioned by various dilutions of H_2SO_4, found no disturbance of normal form in *Coprinus lagopus*, although it had little resistance to desiccation.

A culture technique involving a constantly renewed atmosphere gives a means of controlling independently saturation deficit (and hence transpirational water loss) and the tensions of each of the atmospheric gases. Its application is desirable in this field of work.

In sealed culture vessels both *Collybia velutipes* (Curt, ex Fr.) Quél. and *Polyporus brumalis* (Pers.) Fries. produce stipes, but the pileus of the polypore is absent or abnormal and in the agaric represented by a minute rudiment only. In this paper these and other form-responses are analysed.



TEXT-FIG. 1. Glass culture chamber containing 5 culture flasks. (*a*) Siphons connecting flasks to reservoir (only one is shown in its entirety), (*b*) reservoir of sterile water, (*c*) supply tube containing sterile water, (*d*) and (*e*) clips by which fresh sterile water is let into tube, (*f*) inlet and (*g*) outlet for gas streams.

METHODS

Wide-mouthed 60 ml. flasks (Text-fig. 1) completely full of liquid medium were used for the cultivation of the fungi. This arrangement combined a relatively large volume of medium with a relatively small culture surface which facilitated control of water loss and hastened fruiting. Groups of five such flasks were contained in experimental chambers. These were glass vacuum desiccator jars of standard pattern sealed with a thermostable silicone lubricant (Text-fig. 1).

Each flask was connected by a siphon (*a*) to a central reservoir (*b*) the level of liquid in which was maintained automatically through the tube (*c*) from an external supply of sterile water. The manipulation of two clips (*d* and *e*) on the rubber section of the tube, at intervals of a few days, ensured a column of water in the tube. This emptied itself a little at a time into the reservoir as the liquid surface fell. Cultures would have dried out in a short time if this replacement system had not been employed.

The tubes *f* and *g* permitted the passage of air or other gas mixtures through the chamber. The water-vapour content of the gas mixtures was determined at entry into the chamber and at outflow using a dew-point psychrometer or, in later experiments, a wet and dry junction thermocouple. By difference and knowing the flow-rate (measured by a simple flowmeter at the outlet) water loss from the cultures could be calculated. Trials showed that this depended on the humidity and rate of flow of the air. Temperature being constant, humidity is given as per cent. R.H., which is readily convertible to vapour-pressure deficit, the aspect of atmospheric humidity important for transpiration. Water loss increased smoothly as fruit-bodies developed, due no doubt to their increasing surface.

Fungus surface was estimated for the stage at which mycelium filled the flask mouth just prior to fruiting and again at the end of the experiment. The latter involved a measurement of the overall surface of the fruit-bodies and mycelium. Thus the range of water-loss rates per unit area of fungus during the fruiting period could be calculated. All the flasks were covered with mycelium but a little free water not occupied by the siphons or inlet occurred at the mouth of the reservoir. This was very small and was disregarded in estimating the evaporating surface.

The humidity of gas streams was conditioned by methods based on those of Hopp (1936). Passage through about 50 cm. of anhydrous $CaCl_2$ (10 to 14 mesh) in U-tubes was used for drying. Various humidities were obtained by bubbling through large volumes of saturated solutions of salts in the presence of excess of the solute, diffusers being used to reduce bubble size (cf. Hopp, 1936, p. 29, Fig. 3). A humidifier of Hopp's (p. 33, Fig. 5), basically a water-soaked wick in a glass tower, was modified by a simmerstat-controlled electrical heating tape wrapped round the tower to hasten evaporation. The warm moistened air issuing from it was passed into a large flask in the incubator (which had vigorously stirred air) where it rapidly cooled to ambient temperature before entering the culture chamber. Even by this means it was found difficult to saturate a gas stream completely, although very high vapour-pressures were easily obtained.

Sterilization of gas streams was by aseptic filters of cotton-wool packed into ordinary drying-towers. These were placed immediately before the culture chamber except in the case of very moist gas streams when they were inserted before the humidifier which consequently had to be autoclaved, with all its accessories, at the outset. Liquid medium, of a quantity chosen to avoid overflowing during sterilization, was distributed between the culture flasks and the central reservoir in such a way that the siphons were all full. Its concentration was sufficiently high to allow for subsequent dilution (see below). Entrances to the chamber were plugged with cotton-wool and the whole assembly autoclaved. After cooling the plugs were removed and connexions made with the external supplies with every precaution to ensure asepsis. Passage of dry air through the chamber for a few days removed any condensation, a process which was sometimes hastened by the temporary introduction of dry-heat sterilized containers of anhydrous $CaCl_2$. Finally sterile water was allowed to run into the reservoir until the flasks were full. Medium concentrations given are after dilution.

Inocula were cut with a sterile cork-borer (4 mm. diam.) from a region just behind the margin of vegetative Petri-dish cultures on 2 per cent. malt agar. These usually floated if carefully transferred. Flasks were reinoculated if their first inocula sank during the first 36 hours. Contamination occurred very rarely.

Gas mixtures other than air were supplied commercially in cylinders, their composition being checked by analysis. Laboratory air from a compressor and tank was used. In a few experiments the gas streams were washed through acid permanganate bubblers (1 per cent. $KMnO_4$ in 4 per cent. H_2SO_4), but, as soon as it was evident that no substance affecting the form of the sporophores was thereby removed, the practice was discontinued. The rate of gas flow was controlled by screw-clips on the rubber pressure tubing, a method which appears sufficiently effective with constant ambient temperatures.

For still-air experiments the same kind of chamber was usually employed. Analysis of the final atmospheric composition was by Haldane apparatus. The culture chambers and most of the air-conditioning equipment were kept at constant temperature in a large specially constructed incubator or a constant temperature room, depending on the size of the assembly and on the temperature required. Continuous light was provided by fluorescent-tube lamps, intensity being controlled by adjustment of distance.

Observations on the progress of growth were made through the glass walls of the chambers. At the full term of the experiments, when fruit-bodies had ceased to grow, their numbers and dimensions were sometimes determined.

In the analysis of results means were compared by 'student's' *t* test except in a few cases where a perfectly clear indication of high significance or its lack was given by a simple ranking test (the *U*-test: Mann and Whitney, 1947).

EXPERIMENTS WITH *POLYPORUS BRUMALIS*

1. *Vegetative growth*

Polyporus brumalis was cultivated on approximately 4 per cent. malt extract medium. The suitability of this is probably due to its pH. This was indicated in some preliminary experiments on mycelial growth in malt extract (3 per cent.) buffered with citric acid and Na_2HPO_4 (Table I). The striking optimum in the region of pH 4·0 was also found in a repeat experiment with K_2HPO_4 substituted for Na_2HPO_4 in the buffer, although in this case a minute amount of growth was visible after 15 days in media of pH 6·0 and 7·0. After 6 days, growth on 4 per cent. malt extract was similar at 22° and 26° C., but lower at 18°, 30°, and 34° C.

TABLE I

The Effect of Initial pH of 4 per cent. Malt Extract Medium upon Mycelial Growth of Polyporus brumalis

Temperature 25° C. In darkness
Incubation time 12 days

Initial pH (after autoclaving)	3·2	4·1	5·1	6·0	6·9	7·3
Final pH . . .	3·3	4·4	5·1	6·0	6·9	7·3
Dry weight mycelium (mg.)	11 ± 0·4* (4)†	43 ± 2·7	1·5 ± 0·3	0	0	0

* Mean ± standard error of mean (ϵ); $\epsilon = \sqrt{\left(\frac{\sum (m-M)^2}{N(N-1)}\right)}$.

† In this and subsequent tables the number of replicates is indicated in parentheses.

2. *Preliminary observations on fruiting*

Cultures growing on about 60 ml. of malt extract agar in crystallizing dishes enclosed in a glass-lidded pneumatic trough or sealed completely from the atmosphere in a desiccator jar fruited copiously but abnormally under a variety of uncontrolled light and temperature conditions. Fructifications had reduced caps or curious terminal club-like swellings covered with hymenial tubes (Pl. XXVIII, Fig. 2) or were completely epileate. When caps were lacking the stipes were often of unusual length and strongly phototropic if unilaterally illuminated (Pl. XXVIII, Fig. 1).

In early experiments at room temperature normal pileus formation was not induced in closed cultures either by applying continuous artificial illumination of various intensities, or by daily exposures of 15 min. to weak U.V. light. In other cases the varying of the temperature of unaerated cultures in the light was repeatedly found to yield some caps at 15° C., stipes and club-like abnormalities but seldom caps at 20° C. and no fruits at all at 25° C. This phenomenon is still unexplained although it is certainly not a simple temperature effect for abundant caps are formed at 20 ° C. in renewed air cultures (see below). The probability that a factor of the aeration complex was involved was indicated by some pileate fruit-bodies appearing at 20° C. in pneumatic trough culture chambers which were better ventilated by virtue of wire spacing devices placed between the vessels and their lids.

3. *The effect of evaporating conditions*

Moving-air-stream experiments were first applied to a study of the influence of evaporating conditions.

In the first experiment, at 15° C., the air entering the chambers was varied over the following range of relative humidities (per cent.) by the substances indicated: 0 (anhydrous $CaCl_2$), 33 (saturated $MgCl_2.6H_2O$), 53 (saturated $Ca(NO_3)_2.4H_2O$), 75 (saturated NaCl) and approximately 97 (H_2O). Flow rates were graded from 300 ml./min. for the driest down to 50 ml./min. for the wettest air stream to increase further the differences of water-loss rates resulting. In addition each chamber contained a large surface of the substance with which its air-stream humidity was conditioned. The absorption due to this was not estimated so that absolute rates of water loss could not be calculated in this first experiment.

TABLE II

The Effect of a Range of Evaporation Conditions upon the Ratio : Length of fruit-body (l)/diameter of cap (d), and upon mean numbers of fruit-bodies of Polyporus brumalis

R.H. (per cent.) of ingoing air	0	33	53	75	97
Flow rate (ml./min.)	300	240	180	120	50
l/d	1·5±0·10 (12)	1·7±0·16 (12)	1·4±0·04 (13)	1·6±0·25 (5)	2·9±0·11 (5)
Numbers of fructifications (including rudiments)	9·4±1·8 (5)	16·6±1·8	14·6±2·5	29·2±7·5	1·8±0·2

With the lowest rates of water loss from the cultures stipes tended to be long and caps narrow. The ratio : length of fruit-body (l)/diameter of cap (d) was calculated. This has the advantage of placing emphasis on proportions rather than absolute dimensions. It was significantly higher than in any of the drier chambers ($P=<0{\cdot}01$) which did not differ substantially among themselves (Table II). Also mean numbers of fruit-bodies initiated (count of all sporophores and rudiments) were lower in this wettest chamber than in any of the remainder ($P=<0{\cdot}01$) (Table II). Differences in numbers of initials, barely significant at the 5 per cent. level, also occurred between the chambers with 0 and 75 per cent. and 0 and 33 per cent. R.H. air streams, but in a fashion not confirmed by later work.

An interesting incidental result of this experiment was that it established the xerophytism of developing fruit-bodies of *P. brumalis*. Even in chambers containing a large absorbing surface of anhydrous $CaCl_2$ and with dry air passed into the chamber at the high rate of 300 ml./min. a fine crop developed and shed its spores (morphologically similar groups of fruit-bodies are shown in Pl. XXVII, Fig. 1).

In a second experiment only the extreme (highest and lowest) evaporation conditions of the former series were reproduced, but this time at 20° C. and at 15° C. A uniform rate of flow of 60 ml./min. was employed, but, despite this, the same profound increase of *l*/*d* with diminished water loss was found at both temperatures. The result rules out the remote possibility that flow rate alone might account for the results of the first experiment.

Despite its marked effect, low evaporation seemed not to be associated with the production of the wholly epileate and club-like fruit-bodies of still-air cultures. To find out if atmospheric factors other than humidity were involved several experiments were undertaken but all with negative results (see later sections).

A further trial with a wide range of humidities at 20° C. yielded results consistent with those foregoing in that normally proportioned fruit-bodies appeared in dry-air streams, but, even though flow rates were the same as or even slightly faster than before, the high humidity chambers this time contained completely epileate fructifications. This inconsistency pointed to either an innate variation in the behaviour of the fungus or an unsuspected environmental variable.

Control of light intensity had not been rigorous in these early experiments so that small variations from one to another might have occurred due to altered set-up and ageing of the light source. No actual readings are available but intensities probably ranged between 50 and 70 foot candles.

A further humidity series at much higher light intensity (160 f.c.) showed pilei in all chambers, although the usual relationship between evaporation rate and *l*/*d* was in evidence. Thus it appeared that the failure to obtain consistent responses might be due to the modifying influence of light intensity. This proved to be so.

The interplay of light intensity and rate of water loss

The growth of sporophores at three humidity levels at each of two light intensities was studied. Using a photometer the distance of cultures from a 5 ft. twin fluorescent lamp was adjusted so that one series received approximately 40 and the other approximately 160 f.c. at the level of the mycelium. Temperature was at 20±0·5° C. throughout. The three ingoing air-stream humidities were 0, 75, and 96 per cent. R.H. Flow rates were 200 ml./min. at the first two humidities but slower (5 ml./min.) at 96 per cent. R.H.

Rate of water loss per unit area of the fungus (as previously indicated it is assumed that loss from the tiny exposed water surface of the reservoir is negligible) fell as the fruit-bodies developed and surface increased (Table III). At the time when pileus incepts arose, probably a most significant stage in form determination, it is likely that transpiration per unit area was very near the initial values (*I* in Table III) as surfaces had by then increased little.

As suggested by an earlier experiment, high rates of water loss increase the numbers of fructifications initiated (total count of sporophores, stipes, and rudiments) (Table IV). At 160 f.c. the highest and intermediate rates of

TABLE III

Conditions of an experiment on Polyporus brumalis (*see text*)

I At a stage just prior to fruiting.
F At the end of the experiment.

Light intensity (foot candles)	160						40					
Substance used to condition humidity	Anhydrous CaCl$_2$		Saturated Soln. NaCl.		H$_2$O		Anhydrous CaCl$_2$		Saturated Soln. NaCl.		H$_2$O	
Relative humidity (per cent.) of ingoing air	0		75		96		0		75		96	
Flow rate of air (ml./min.)	200		200		5		200		200		5	
	I	*F*	*I*	*F*	*I*	*F*	*I*	*F*	*I*	*F*	*I*	*F.*
R.H. of outgoing air	69·5	85	88	93	96·5	96·5	66·5	83	88	93	96·5	96·5
Rate of water-loss from cultures (mg./hr.)	152·91	194·85	27·60	38·21	0·079	0·081	147·03	184·42	27·60	37·93	0·079	0·078
Surface area of fungus (cm.^{2})	22·62	118·81	22·62	63·69	22·62	61·97	22·62	88·24	22·62	52·68	22·62	52·08
Estimated rate of water-loss by fungus (mg./cm.^{2}/hr.)	6·76	1·64	1·22	0·60	0·0035	0·0013	6·50	2·09	1·22	0·72	0·0035	0·0015

water loss (for actual values see Table III) were associated with similar mean numbers per flask and a large significant drop occurred with further diminution of transpiration ($P=<0{\cdot}05$). The additive effect of light and humidity treatments was shown by the shift of this sharp change to the step from driest to intermediate air conditions at 40 f.c. ($P=<0{\cdot}01$).

TABLE IV

Effect of Light Intensity and Rates of Water Loss from Cultures of Polyporus brumalis *upon Mean Numbers of Fructifications (including rudiments) and of Pileate Fructifications per Flask*

	Light Intensity (foot candles)					
	160			40		
Rate of water loss*	High	Intermediate	Low	High	Intermediate	Low
No. of fructifications (including rudiments)	14·6±4·2 (5)	16±3·8	6·2±1·8	11·2±2·2	4·2±0·3	5±0·9
No. of pileate fruit-bodies	3·2±0·8	1·2±0·7	0·8±0·5	2±0·4	0·4±0·3	0

* See Table III.

Mean numbers of pileate fructifications fell with decreasing transpiration rates (Table IV, Pl. XXVII). Differences were significant between the least or intermediate rates and the highest ($P=<0{\cdot}05$ at 160 f.c. and $<0{\cdot}01$ at 40 f.c.). No fruit-bodies produced caps in the weaker light at the highest humidity.

TABLE V

Effect of Light Intensity and Rates of Water Loss from Cultures of Polyporus brumalis *upon Average Time for Onset of Fruiting and for Initiation of Pilei (Days after Inoculation)*

	Light Intensity (foot candles)					
	160			40		
Rate of water loss*	High	Intermediate	Low	High	Intermediate	Low
Appearance of rudiments	6·13±0·09 (15)			7·87±0·35		
Appearance of 1st pileus of flask	8·8±0·9 (5)	13±0·9 (4)	16±0 (3)	11±1 (3)†	18±0 (2)	>25

* See Table III.
† 2 late abnormal fructifications ignored.

Cultures at 40 f.c. were collectively 1 to 2 days later in the production of the first visible fruit-body rudiments than those in strong light ($P=<0{\cdot}01$) (Table V). Evaporating conditions were without influence.

Time of pileus initiation (time of appearance of the first pileus on each flask) was, in contrast, delayed by each reduction of evaporation ($P=<0{\cdot}05$). The effect was more marked at 40 f.c. In the extreme case, with least water loss at the lower light intensity, cap production did not occur in the term of the experiment (Table V, Pl. XXVII, Fig. 6) and as growth of the stipes had apparently ceased, would almost certainly never have occurred.

The tendency for conditions of low evaporation to promote the continued growth of the stipe without cap formation is brought out very well by the figures (Table VI) for length of stipe at the time of the apical flattening which heralds pileus formation (this appearance is seen in Pl. XXVII, Fig. 5). These measurements had, unfortunately, to be made from outside the chambers during growth, but are, nevertheless, probably reasonably accurate. This dimension increased rapidly with each diminution in rate of water loss at both light intensities ($P = < 0{\cdot}01$). A similar result accompanies reduction of light intensity at each transpiration value where data are available ($P = < 0{\cdot}01$).

Even when stipes were epileate growth did not continue indefinitely although some approached 60 mm. in length in the moister chambers. Whether growth cessation was due to innate characteristics or to a limitation imposed by the set-up is not known.

As the pileus widens stipes continue to elongate (although perhaps by another growth mechanism—see discussion). This is shown by a comparison of mean lengths of sporophores at the time of pileus initiation and at maturity (Table VI). The increment in length after cap initiation expressed as a percentage of the length at initiation (Table VI) does not differ between the highest water loss sample at 160 f.c., the intermediate sample at 160 f.c. and the highest transpiration sample at 40 f.c. These, however, show an enhanced percentage elongation as compared with pileate fructifications in the remaining cultures ($P = < 0{\cdot}05$). The two very long stipes which grew in the intermediate chamber at 40 f.c. produced their minute pilei (Pl. XXVII, Fig. 5) as a final growth activity.

Whatever proportions of their overall lengths are attributable to growth before or after cap initiation, the final dimensions of pileate fruit bodies were strikingly dependent on the external conditions (Table VI). Thus at 160 f.c. their lengths increased with each reduction of transpiration rate ($P = < 0{\cdot}01$) and at 40 f.c. from the highest to the intermediate rates ($P = < 0{\cdot}05$). Also the two less humid chambers in stronger light contained shorter pileate fruit bodies than their counterparts in weaker light ($P = < 0{\cdot}01$). With the high or intermediate water-loss samples at 160 f.c. as reference points, reduction of transpiration rate is associated with a very similar effect to reduction of light intensity.

Variability made the data of cap diameters a less sensitive index of the evaporation and light influences than stipe length. At the higher light-intensity cap diameters in the highest and intermediate water-loss chambers were not distinguishable upon analysis, but they fell sharply in the wettest chamber ($P = < 0{\cdot}02$) (Table VI). In weaker light visual indications (Pl. XXVII, Figs. 4 and 5) would lead to the expectation of a decided drop in cap size from the high to the intermediate rates of water-loss. Owing, however, to the small size of one of the samples, statistical significance was not obtained unless three atypical fruit-bodies with rudimentary pilei in the driest sample were ignored, when the value of $P=<0{\cdot}02$. Cap diameter was zero in the wettest chambers. Wider caps showed at 160 f.c. than at 40 f.c. at the intermediate level ($P=<0{\cdot}01$) and at the high transpiration level if the three anomalous fruit-bodies were ignored ($P=<0{\cdot}01$).

The data for stipe length and cap diameter can be combined strikingly by calculating l/d. This coefficient (Table VI) rose steeply with each reduction of transpiration or light intensity where comparison was possible ($P=<0{\cdot}01$).

TABLE VI

Effect of Light Intensity and Rate of Water Loss from Cultures of Polyporus brumalis *upon Dimensions (mm.) of Fructifications and Related Values*

	Light Intensity (foot candles)					
	160			40		
Rate of water loss*	High	Intermediate	Low	High	Intermediate	Low
Length of stipe at cap initiation (l^1)	7·4±0·7 (16)	12·5±0·9 (6)	31·7±8·6 (4)	12·1±1·1 (10)	58·5±2·3 (2)	..
Length of pileate fructifications (l)	20·8±0·9	32·5±7·9	48·7±4·5	30·6±9·1	58·5±2·3	..
Increase in stipe length after cap initiation, per cent. $\left(\frac{l-l^1}{l^1}\times 100\right)$	206±21	172±37	65±18	161±25	0	..
Cap diameter (d)	15·8±1·3	14·3±1·7	7·5±0·6	12±2·8	2·0±0·5	0
l/d	1·4±0·1	2·3±0·1	6·6±0·8	6·5±2·6	30·8±6·4	..
Length of epileate fruit-bodies	7·5±1·1 (8)	13±1·7 (11)	24·7±3·6 (13)	10·3±0·7 (7)	31·9±4·5 (12)	33·9±5·0 (14)

* See Table III.

It should be noted that l/d cannot be calculated for fruit-bodies of the dampest chamber at 40 f.c., since they all lacked pilei and values of l/d in this paper are based only on fructifications with definite caps.

Small numbers of abortive epileate fructifications occurred in all chambers. In drier air these tended to shortness and doubtless had been potentially pileate, unlike vigorously growing stipes in the moister chambers which were inhibited from cap production by conditions of low evaporation. Thus the effect of considering the final crop of epileate fructifications exceeding 5 mm. in length (all shorter were excluded as primordia) was to show an increase in mean length with diminishing rate of water loss (Table VI, Pl. XXVII). This was significant at each stage at 160 f.c. and from high to intermediate levels at 40 f.c. ($P=<0{\cdot}05$). The final reduction of water-loss rate at the low light intensity produced no further meaningful change. Here, as suggested previously, maximum stipe length in the given experimental assembly may be limited by factors other than those under investigation.

Stipe diameter was a very variable feature of fruit-bodies in most samples. Nevertheless the only 10 stipes having a diameter at the middle of 3 mm. or more (all pileate sporophores) were confined to the two chambers into which dry air was passed (Pl. XXVII).

The following arguments may be adduced against the possibility of flow-rate having a direct effect in this experiment; firstly, major changes in response, as in a previous experiment, occurred without change of flow-rate between the driest and intermediate chambers at both light intensities, while there are only slight differences at 40 f.c. between the intermediate and wettest chambers despite a flow-rate difference. Secondly, in other experiments epileate fruit-bodies alone have been obtained at flow-rates varying from 5 to 150 ml./min. given low light intensity and low vapour-pressure deficits. Thirdly, an *a priori* basis for flow-rate action is hard to find. A mechanical effect is utterly unlikely at the relatively slow rates used, and even at the slowest rate analysis showed the gaseous composition of the culture atmosphere not to differ measurably from air.

4. *The effect of darkness*

In view of the action of light shown in the last experiment it is of interest to consider the effect of zero light intensity upon sporophore form. This was shown in an experiment which also indicated that humidity was the only important atmospheric factor acting in the conditions of the experiment.

In this experiment two of four chambers were aerated with dry air streams (40 ml./min.) and two had unrenewed air. One of each kind was enclosed in a specially constructed wooden box. The entry points of the air and water-supply and air-outflow tubes, like the rest of the box, were rigorously light-tight. The remaining cultures received light of approximately 60 f.c. intensity, and all remained at 20±0·5° C. throughout. Chambers contained, below the shelf, a large surface of initially anhydrous $CaCl_2$, so that high transpiration conditions obtained in all of them. This would be slightly higher in the renewed air chambers. The unilluminated cultures were opened only when development was complete in the ones in light (24 days after inoculation). The striking results are seen in Pl. XXVIII, Figs. 3, 4, 5, 6.

Although it did not entirely prevent initiation, darkness greatly diminished numbers of fructifications (including rudiments) ($P = < 0{\cdot}01$) (Table VII), a result consistent with the action of reduced light intensity on numbers in the last experiment. Renewal of air seemed without influence on numbers of incepts in light or darkness.

Most spectacular of all was the evidence of the entire dependence of pileus formation upon light (Table VII and Pl. XXVIII, Figs. 3, 4, 5, 6) even under optimal transpirational conditions.

In addition a distinct effect upon stipe form was present. The unusually pale columnar outgrowths of darkened cultures were stout and blunt. The apices in some of the aerated samples had swollen out in a fashion suggesting vegetative reversion and on the swellings one or several new primordia had arisen. Thus branching was simulated. The distal proliferation in no way resembled pilei either in shape or in bearing pores (Pl. XXVIII, Fig. 4). The tendency of reduction of light intensity to increase stipe length seen in the previous experiment was not carried farther by total exclusion of light. Indeed stipes in the darkened cultures appeared rather short, at any rate at the time of harvesting. Their stoutness, however, was suggestive of a measure of light control of stipe girth in normal illuminated cultures.

Differences between stagnant- and renewed-air cultures were slight. In the light mean numbers of pileate fruit bodies were greater in moving air ($P = < 0{\cdot}01$) (Table VII) and the fructifications appeared slightly shorter than in still air (Pl. XXVIII, Figs. 5, 6), although data were not collected on this point. Hymenial tubes appeared better developed in the aerated chamber, but both samples sporulated copiously. These features might be related to the slightly higher transpiration rate of the fresh air culture (but see the effects of CO_2, below).

The two light cultures were outstandingly similar. The paramount factor of transpiration rate having been controlled (by anhydrous $CaCl_2$), any other atmospheric factor influencing form was free to show itself in the still air culture, but failed to do so. A final CO_2 concentration of 6·6 per cent. was measured in this chamber.

TABLE VII

The Effect of Light and Darkness and Renewal and Non-renewal of Culture Atmosphere upon Mean Numbers of Fruit-bodies (and Rudiments) Initiated, and upon Mean Numbers of Pileate Fruit-bodies of Polyporus brumalis

	Light		Dark	
Culture atmosphere	Renewed	Still	Renewed	Still
Pileate fruit-bodies	5±1·1 (5)	2±0·4	0	0
All fructifications	15±2·9	11·6±1·0	2·2±0·2	2±0

5. *Factors not active, and club-shaped fruit bodies*

Certain atmospheric factors were shown not to influence fruit-body form in a number of experiments which are now briefly mentioned.

In one series of tests gas mixtures like air but with CO_2 enriched at the expense of nitrogen were passed at various speeds and with various water-vapour contents over growing cultures. Concentrations of CO_2 up to 5 per cent. failed to cause differences from controls in moving air of the same relative humidity and flow rate. Some fruit-bodies in 5 per cent. CO_2 had a poorly developed hymenial tube layer, recalling the similar condition in the illuminated but unaerated sample in the last experiment. Too much weight should not, perhaps, be attached to a single observation of this type.

In another series chemical reagents were introduced by various means into till-air culture chambers to serve as absorbents of hypothetical atmospheric staling agents. Daily opening of a valve for a few moments prevented abnormal pressures developing. Comparisons with still and moving air controls lacking absorbents were made. Neither KOH (5 per cent.), nor $KMnO_4$ (1 per cent. in 4 per cent. H_2SO_4) nor the presence of both these reagents did anything to prevent the appearance of the usual abnormalities. Thus CO_2 and a very wide range of other volatile substances were excluded from activity.

Anhydrous $CaCl_2$, alone or when introduced simultaneously with the other reagents, always ameliorated the abnormalities characterized by high l/d ratios, but did not prevent the appearance of 'clubs'. These were, however, often very few in number in still-air chambers (see again the illuminated unaerated sample of the experiment described in the last section, Pl. XXVIII, Fig. 5).

The club-like abnormality was a feature of the relatively dry chambers. It could occur in the absence of CO_2 and, very rarely, even in moving air chambers. The still-air experiments were run at fairly low light intensity (*c.* 60 f.c.) and not infrequently the final oxygen content of the chambers was less than 1 per cent. Further work on the 'club' abnormality is needed (see discussion).

EXPERIMENTS WITH *COLLYBIA VELUTIPES*

Collybia velutipes was cultivated like the polypore but upon a chemically defined medium (Plunkett, 1953) in which the sucrose concentration after topping-up flasks was 3 g./100 ml. Incubation temperature was 20±0·5° C. and illumination continuous at an intensity of about 70 f.c. Under the conditions described this fungus proved more variable in time of fruiting and in numbers and dimensions of sporophores produced than *P. brumalis*, so that a less detailed analysis of its fruiting response was achieved, and the results are of a more preliminary kind.

1. *The effect of evaporating conditions*

Air streams flowing at 200 ml./min. into culture chambers were conditioned to the following relative humidities with the substances indicated: 0 (anhydrous $CaCl_2$), 20 (saturated K acetate), 53 (saturated $Ca(NO_3)_2$, $4H_2O$), 86 (saturated KCl), approximately 97 (H_2O). Clusters of pileate rudiments developed under all conditions, but only in the two most humid air streams did fruit-bodies complete elongation of stipes and expansion of caps. In the 53 per cent. R.H. stream, in which transpiration never exceeded 1·4 mg./cm.^{2}/hr., and in the drier air streams fruit-bodies ceased to grow when not more than 1 cm. in length, before stipe or cap expansion had progressed far. However, they were normal up to the time of growth cessation, no differential effect on cap and stipe being evident. Unlike the sporophores of *P. brumalis* those of *C. velutipes* are prevented from expansion by rates of water loss exceeding quite low values and show no modification of form in relation to this factor.

2. *Atmospheric staling in still air*

Next cultures in a still-air chamber which also contained a large absorbing surface of $N/_1$ KOH were compared with a control lacking KOH and another having slowly renewed moist air (5 ml./min.). All the chambers showed much condensation on the walls indicating a high atmospheric humidity favourable to fruit-body development. Normal KOH tends to come into equilibrium with an atmosphere only slightly less than saturated.

The pileus rudiment in *C. velutipes*, like that of most agarics, is laid down at a very early stage of development when the fruit-body is 2–3 mm. high (Plunkett, 1953, Pl. V, Fig. 2). In moving air (Pl. XXIX, Fig. 3) and in still air over KOH (Pl. XXIX, Fig. 2) the stipes and cap rudiments expanded together in the normal way. In still air alone the stipes expanded vigorously but the pileus initials remained as tiny apical rudiments (Pl. XXIX, Fig. 1).

The moving air treatment was applied after the sporophores shown in Pl. XXIX, Fig. 3 had started to grow and the sudden increase in thickness which they show about half-way along the mature stipe marks its effect. This is of the highest interest as it suggested that thickening of the stipe may, like pileus expansion, have a sensitivity to external factors different from stipe elongation.

It is clear from this experiment that KOH can remove an atmospheric staling factor from still air cultures which is inhibitory to pileus development.

3. *The effect of carbon dioxide concentration*

Acting on the hypothesis that the volatile substance removed by KOH was CO_2, experiments were established in which gas mixtures resembling air but enriched in CO_2 at the expense of N_2 were passed over cultures at a rate of 20 ml./min. In all cases humidity was controlled at an undetermined but high uniform level by bubbling through water. While this treatment increased their vapour pressure it was shown by analysis that after the first few hours it had no effect on the proportions of the other gases in the mixtures. Still and moving air controls were used.

For unknown reasons fruit-body numbers were rather variable even within a sample. To exclude numerous small abortive fructifications, apparently unrelated to the consciously varied aeration factors, only those exceeding the arbitrary length of 20 mm. were considered in the comparisons which follow.

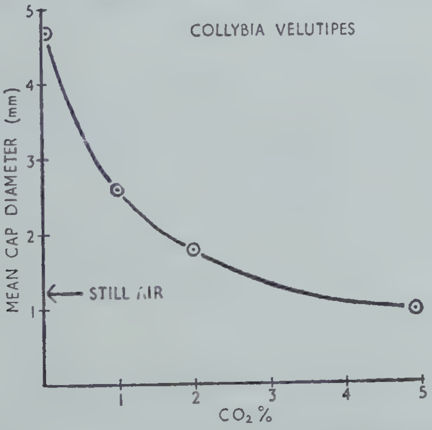
The most striking data were provided by cap dimensions (Table VIII, Text-fig. 2). The increase in concentration of CO_2 from atmospheric level (analysis of laboratory air gave 0·06 per cent.) to 1·0, 2·0, and 4·9 per cent. was associated with marked reduction of mean pileus diameter at each stage ($P = < 0{\cdot}01$), only tiny rudiments of caps occurring at the highest concentration (Pl. XXIX, Fig. 5). The still-air cultures had caps of similar mean diameter to those in 4·9 per cent. CO_2, which was in fact approaching the concentration (6·8 per cent.) found finally in the staled atmosphere. It was noted visually that caps expanded progressively less as this sample aged, presumably because of rising CO_2 tension.

Stipes were significantly shorter in 4·9 per cent. CO_2 than in moving air or 1 per cent. CO_2 ($P = < 0.01$). Pl. XXIX, Fig. 4 shows fructifications from the 1 per cent. CO_2 gas stream, which often had very long stipes, with, however, always some reduction of pileus development (such caps never flattened). An expected enhancement of stipe elongation by this low CO_2 concentration as compared with air (see discussion) was not proven by the data. Stipe diameters were not measured, but the stout stipes found in some fruit-bodies in moving air and in 1 per cent. CO_2 were lacking in 4·9 per cent. CO_2 (Pl. XXIX, Fig. 5). The highest CO_2 and still-air treatments often caused blackening of fruit-bodies.

TABLE VIII

The Influence of CO_2 Concentration in Air-like Gas Streams upon Mean Pileus Diameter (mm.) of Collybia velutipes.

Moving air (c. 0·06% CO_2)	1% CO_2	2% CO_2	4·9% CO_2	Still air
4·7±1·2 (8)	2·6±0·16 (39)	1·8±0·15 (30)	0·96±0·09 (25)	1·21±0·09 (82)



TEXT-FIG. 2. The effect of CO_2 concentration in air-like gas streams upon mean cap diameters of *C. velutipes*.

The results obtained were consistent with the supposition that CO_2 is the pileus-inhibiting agent removable by KOH in still-air cultures.

4. *The validity of the supposed morphogenic effect of light*

Elsewhere it has been shown that fruit-bodies of *C. velutipes* developing in light-tight boxes lack expanded pilei (Plunkett, 1953, Pl. V, Fig. 4), and it has been attributed to the effect of light-exclusion. The discovery of similar inhibition by atmospheric staling raised the question of the validity of the supposed dark effect as insufficient aeration might have been responsible. This question is of especial interest as Lakon (1907) in a study of a coprophilous *Coprinus sp.* claimed that poor cap development could equally well be prevented by illumination or by aeration.

As in an experiment with *P. brumalis* (see above) still- and renewed-air cultures in light were compared with a similar pair of chambers in rigorous darkness. Atmospheric humidity was as usual controlled at a favourable high level. The dark cultures were opened 40 days after inoculation when fruiting was complete in the illuminated controls.

The results were quite unequivocal (Pl. XXIX, Figs. 6, 7, 8, 9). Only in illuminated aerated cultures were expanded caps produced. Clearly in this fungus both light and absence of atmospheric staling are necessary if pileus expansion is to occur.

The mycelium in dark, still cultures was white. Either aeration or light caused a pale brown pigmentation and this intensified if both conditions operated. To some extent young fruit-bodies behaved similarly, though in old fructifications a dark brown colour tended to develop irrespective of treatment.

Discussion

The discovery of another agaric, the development of which is disturbed by quite low concentrations (1 per cent.) of CO_2 lends some indirect support to Lambert's (1933) findings with the cultivated mushroom. This is of interest as his work might have been called into question on grounds of procedure following the demonstration of other volatile factors producing mushroom abnormality (Mader, 1945; Stoller, 1952). In *Collybia velutipes*, as in the mushroom, higher CO_2 concentrations (*c.* 5 per cent.) were necessary for stipe than cap retardation.

The control of fruit-body form in *Ganoderma applanatum* by atmospheric humidity (Hopp, 1938) takes on new interest now that similar sensitivity is known in another polypore.

The principal fungus responses studied in this communication are cap and stipe development. It should be realized that these probably represent different types of growth in the agaric and the polypore, and even in one fungus at different times. Thus the expansion of the early-formed pileus rudiment and the stipe in *C. velutipes* is possibly by indirect growth, that is by a method involving change of size or arrangement of pre-existing cells [cf. *Collybia apalosarca* (Corner, 1934), *Coprinus lagopus* (Borriss, 1934*a*)]. Conversely in *Polyporus brumalis* cap expansion (which it has been shown possible to postpone indefinitely) and stipe elongation prior to cap formation is possibly by direct growth, that is, apical extension of hyphae and increase in cell numbers with or without some immediately subsequent cell enlargement [cf. *P. xanthopus* (Corner, 1932), other polypores (Corner, 1953) and *Hygrophorus firmus* (Corner, 1935)]. Following cap delimitation, however, stipe growth must be indirect. Furthermore it should not necessarily be assumed that growth of a structure is by the same mechanism under all experimental conditions. Hyphal analysis of developmental stages of normal and abnormal fruit-bodies is required to settle the problems raised by this discussion.

In *P. brumalis* reduction of transpiration rate was associated with a fall in the percentage increase in stipe length after cap initiation, despite the stipes greater overall length. Among various possibilities are the following: (*a*) that a direct action of low transpiration on growth after cap initiation was involved, (*b*) that only a limited apical region of the stipe can show this sort of growth (growing stipes have white sappy distal and brown fibrous proximal portions), (*c*) that an unidentified factor (? nutritional) might limit the stipe length attainable (even epileate stipes were not of unlimited growth in the conditions of my experiments).

The enhanced stipe length of *P. brumalis* fruit-bodies lacking or with reduced pilei given low transpiration or low light intensity (but, strangely, not in darkness) is well established. This may be the expression of an independent action of external conditions on stipe and cap. Alternatively, however, it may reflect a high degree of integration of the developmental processes of the two structures. This might be visualized in terms of a restraint on stipe elongation produced by the pileus in proportion to the vigour of its development. Magnus (1906) and Schenck (1919) have hinted at a negative correlation of stipe and cap growth in certain agarics. Spectacularly long stipes associated with small or absent caps have not infrequently been recorded as in *Coprinus stercorarius* (Brefeld, 1877), *Lentinus lepideus* (Buller, 1905) and *Polyporus squamosus* (Buller, 1906). Very long fruit-bodies of *C. velutipes* found growing in a shed by Sowerby (Biffen, 1898) were perhaps of this kind. My experiments did not prove increased stipe elongation associated with cap retardation in *C. velutipes* but further work is desirable

Whether or not the developmental state of the cap may affect stipe growth it is clear that the processes underlying fruit-body development have different physiological tolerances. In particular those responsible for cap production (*P. brumalis*) and expansion (*P. brumalis* and *C. velutipes*) emerge as highly sensitive. For *P. brumalis* data of time of cap initiation, length of stipes at cap initiation, lengths and numbers of pileate and epileate fructifications and cap diameters point to a strong tendency for pileus suppression while stipe elongation is, if anything, promoted when transpiration and light intensity are reduced. Similarly in *C. velutipes* darkness or low CO_2 concentrations (1–2 per cent.) restrain cap expansion without preventing stipe elongation. This is essentially the finding of some workers on the light relations of developing sporophores (Brefeld, 1877; Buller, 1905, 1906; Lakon, 1907; Schenck, 1919; Galleymore, 1949).

It is a remarkable fact that the same morphological response of a fruit-body is sometimes evoked by different stimuli. Thus light or heightened temperature (*C. stecorarius*, Brefeld, 1877; Grantz, 1898) or either of these or mechanical stimulation (*C. lagopus*, Borriss, 1934*b*) seem largely interchangeable. In the latter fungus Stiefel (1952) has shown that although light and mechanical stimulation must be perceived differently they probably cause an identical excitation. In *P. brumalis* high light and high transpiration appear to act differently. They are additive in effect rather than substituting for one another. Fruit-bodies are epileate in darkness irrespective of high transpiration, and high light did not result in normal fruit-bodies if vapour-pressure deficits were low. The positive stimulation of light and negative stimulation (? toxicity) of CO_2 for *C. velutipes* are clearly not substitutes. It would be premature to consider whether single or multiple stage processes are being affected in these two fungi.

The observation has often been made that light-starved agaric fruit-bodies have slender stipes (e.g. Biffen, 1898, for *C. velutipes*). In *C. lagopus* Stiefel (1952) was able to use increase of stipe diameter as an index of the morphogenic effects of light and mechanical stimulation. In harmony with these cases are the preliminary indications of increase in stipe diameter with removal of atmospheric staling (*C. velutipes*) and increased transpiration (*P. brumalis*). Conversely, however, stipes of *P. brumalis* appeared stouter in darkness.

The complex problems relating to the primary initiation of fruit-bodies on the mycelium have been little studied in hymenomycetes. In both *C. velutipes* and *P. brumalis* some primordia arose in total darkness as in certain *Coprinus* spp. (Borriss, 1934*b*; Schenck, 1919) but not *Bolbitius* spp. (Schenck, 1919). However, in *P. brumalis* numbers of incepts were greater in light and at higher rates of transpiration. An earlier and numerically greater response likewise occurred with rising light intensity. A dependence of the time of fruiting in *C. velutipes* on carbohydrate concentration of the medium has already been shown (Plunkett, 1953).

The club-like abnormality of fruit-bodies of *P. brumalis* has not been produced at will, although it arose very rarely in moving air and never in brightly illuminated aerated cultures. It was not caused by CO_2 and transpiration conditions, but it was never present in cultures in which caps were totally inhibited by low vapour-pressure deficits. Tentatively it might be regarded as an abortion just prior to cap formation. Such would occur occasionally under a wide range of conditions but might be expected frequently in still-air cultures where abortion is common. Extreme depletion of O_2 which may occur in such cultures and low light intensity should perhaps not be disregarded in considering the causes of abortion.

P. brumalis has been shown to expand its fruit-bodies freely at rates of water loss at least six times greater than those preventing normal expansion in *C. velutipes*. This is associated with a tougher sporophore construction in the polypore. Perhaps the fact that *P. brumalis* may be collected at all times of the year (Heim and Cailleux, 1953) whereas *C. velutipes* is usually a 'winter-fungus' in temperate climates (Buller, 1924, p. 489) may not be unrelated to the degree of xerophytism of the developing fruit-bodies.

The caps having a greater sensitivity to external conditions than the stipe may be of importance in nature. Thus stipes developing in recesses of the substratum might because of low light intensity, atmospheric staling or low vapour-pressure deficits remain without, or with only rudimentary, pilei until growth had carried the apices into the light or into fresher or drier air. This could be a device tending to ensure spore liberation under conditions favourable to dispersal. Its success would depend on the stipe's capacity to grow to unusual lengths (see above) and on its tropisms. Positive phototropism is established in *P. brumalis* and probably occurs in the agaric. 'Aerotropisms' should be sought experimentally. There is as yet no direct evidence to support this hypothesis.

The work described has certain implications for mycological technique. If fructification of hymenomycetes (and possibly other fungi) is to be studied in pure culture it is evident that regard must be had to the atmospheric environment as well as nutritional and other familiar factors.

The value of renewed atmosphere technique for the separation of 'aeration' factors is shown. Thus the response of *P. brumalis* to evaporation conditions was demonstrated at normal atmospheric tensions of CO_2 and O_2. In *C. velutipes* a reaction to CO_2 was shown at normal O_2 concentration with humidity not limiting. Attention may also be drawn to the matter of technique in investigating fungus responses to light. In the present work the clearly demonstrated effect of light intensity upon sporophore development in *P. brumalis* was completely missed in some preliminary trials (see above) because of the masking effect of still air conditions. Again, the obtaining of rigorous darkness should never lead to poor ventilation.

SUMMARY

The mycelium of *Polyporus brumalis* on malt-extract medium has growth optima in the region of pH 4·0 and between 22 and 26° C.

Fructifications arising in still air chambers at 20° C. lack caps or show reduced pilei or terminal swellings of the stipe. Moving sterile air streams of known gaseous composition and vapour pressure, controlled temperature and illumination, and automatic replacement of water loss from the medium were employed to analyse the abnormalities.

Reductions of transpirational water loss over the approximate range of 6·5 to 0·0015 mg./cm.^{2}/hr. at normal atmospheric levels of O_2 and CO_2 were associated with (1) delay of pileus production, (2) increased mean lengths of stipes at pileus initiation, (3) diminished numbers of pileate fructifications, (4) increased final lengths of pileate and epileate fruit-bodies, and (5) diminished cap diameter. A very similar but less marked effect was obtained by reduction of light intensity from 160 to 40 f.c. at each transpiration level studied. The effects were additive so that with least light and lowest transpiration pileus suppression was complete in all fructifications. Total darkness prevents cap development even in dry moving air.

Fewer fruit-bodies are initiated when transpiration rate is low and when light intensity is zero or at low values. Low light intensity also delays fruiting.

The abnormalities in still-air chambers are not prevented by treatment of the atmosphere with KOH or acid K permanganate nor do concentrations of CO_2 up to 5 per cent. in air-like gas mixtures affect fruit-body development.

Collybia velutipes has only rudimentary pilei in unrenewed air cultures. The atmospheric staling agent responsible may be removed with KOH. A significant reduction of cap diameter with increasing concentration of CO_2 in air-like gas streams from atmospheric level up to 5 per cent. occurs. 1 per cent. of CO_2 produced a measurable effect.

The validity of an earlier report of the dependence of cap expansion upon light is established by carefully controlled separation of the light and aeration factors.

The *C. velutipes* fruit-body does not show form responses to changed transpiration rate, but is arrested before it exceeds 1·4 mg./cm.^{2}/hr. *P. brumalis* fruit bodies on the other hand are relatively xerophytic, developing at rates probably in excess of 9·0 mg./cm.^{2}/hr.

Preliminary indications are noted that *P. brumalis* may respond to low transpiration and *C. velutipes* to high CO_2 by a diminution of stipe girth.

The results are discussed from the standpoints of mycological technique, fruit-body development and significance in nature.

ACKNOWLEDGEMENTS

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DESCRIPTION OF PLATES

Illustrating B. Plunkett's article 'The Influence of Factors of the Aeration Complex and Light upon Fruit-Body Form in Pure Cultures of an Agaric and a Polypore'.

The smallest divisions of the scale lines on the plates all represent 1 cm.

PLATE XXVII

P. brumalis:

The total crop of fructifications from an experiment in which light intensity and transpirational water loss were varied.

1. 160 foot candles, 6·76—1·64 mg./cm.^{2}/hr. Normally proportioned fruit-bodies.
2. 160 f.c., 1·22–0·60 mg./cm.^{2}/hr.
3. 160 f.c., 0·0035–0·0013 mg./cm.^{2}/hr.
4. 40 f.c., 6·5–2·09 mg./cm.^{2}/hr.
5. 40 f.c., 1·22–0·72 mg./cm.^{2}/hr. Note pileus initials on 2 fruit-bodies.
6. 40 f.c., 0·0035–0·0015 mg./cm.^{2}/hr. Epileate fruit-bodies.

PLATE XXVIII

P. brumalis:

1. Positively phototropic epileate stipes from still air cultures.
2. Club-shaped abnormalities, and one abnormal pileus, from still air cultures. Note tube layer on swellings.

3–6. The total crop of sporophores from an experiment with still and renewed air conditions in darkness (black background) and light (white background). Transpiration rate was high in all cases.

3. Dark, unrenewed air.
4. Dark, renewed air.
5. Light, unrenewed air. (Note a few club-shaped sporophores.)
6. Light, renewed air.

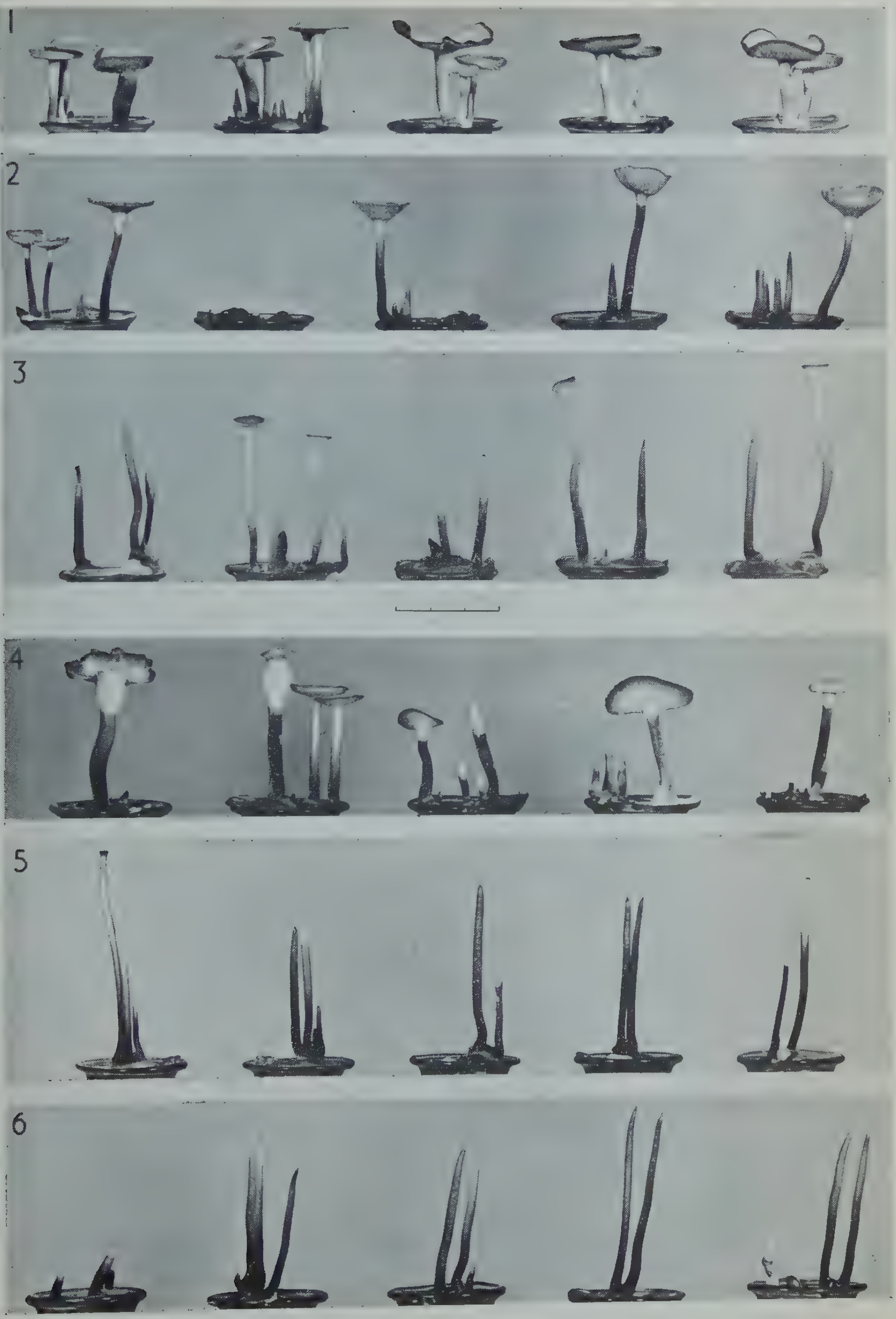
PLATE XXIX

Collybia velutipes:

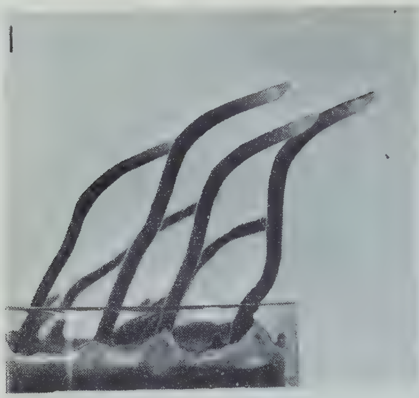
1–3. Representative fruit-bodies from parallel cultures as follows: (1) In still air, (2) In still air over KOH, (3) In moving air. The increase in thickness of stipes in 3 occurred when the moving air conditions were applied.

4 and 5. Fruit-bodies grown in air-like gas streams containing 1 per cent. (4) and 5 per cent. (5) of CO_2.

6–9. Representative fruit-bodies from an experiment with still and renewed air in darkness (black background), and light (white background): 6 Still air, darkness; 7 renewed air, darkness; 8, still air, light; 9, renewed air, light—only in this case were pilei expanded.



B. E. PLUNKETT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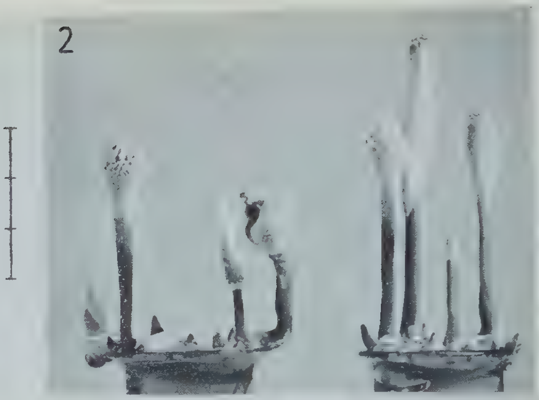
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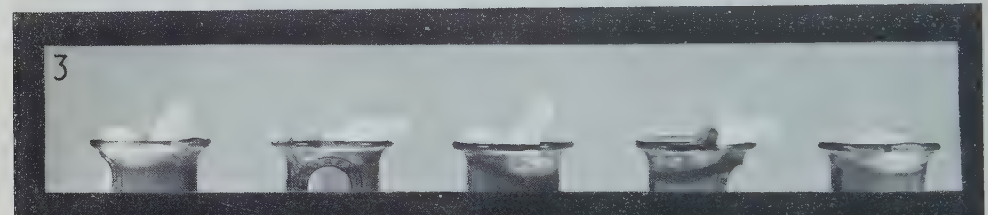
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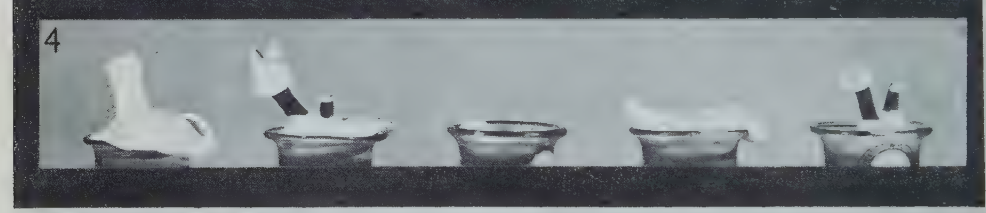
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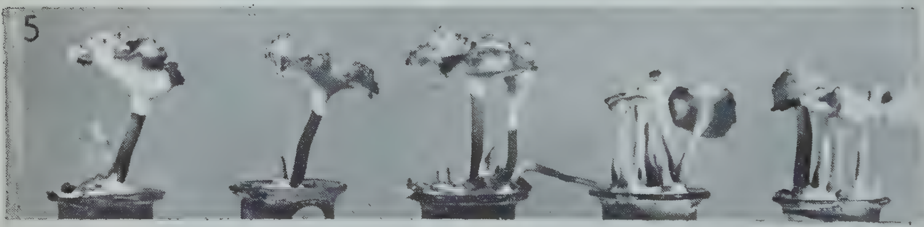
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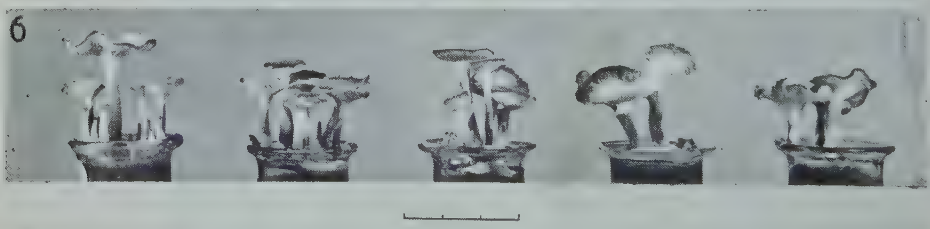
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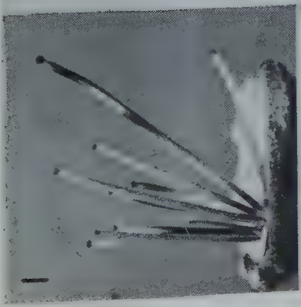
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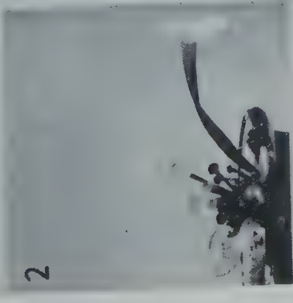
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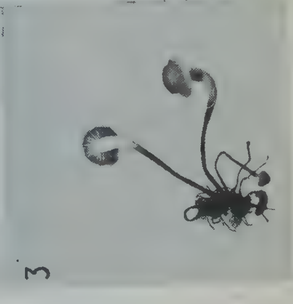
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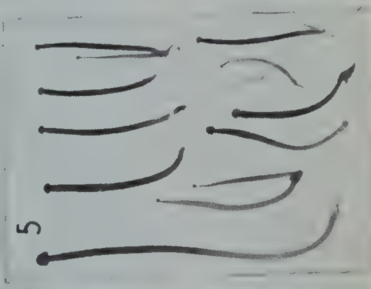
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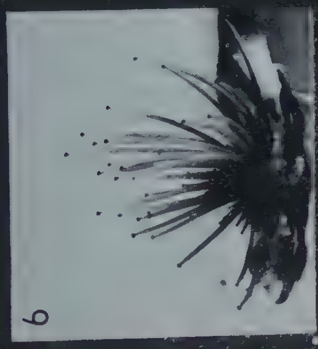
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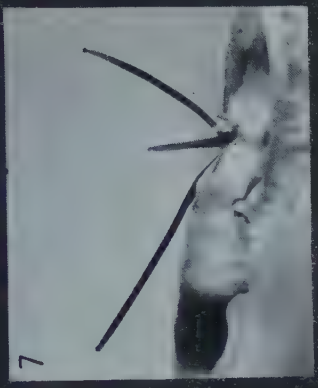
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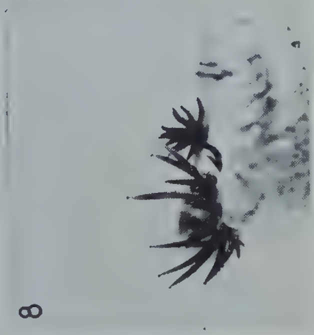
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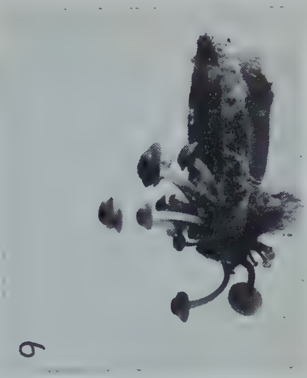
STILL AIR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RENEWED AIR

B. E. PLUNKETT

Effects of Natural and Artificial Light in Arctic Latitudes on Long- and Short-day Plants as Revealed by Growth Analysis

BY

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With six Figures in the Text

ABSTRACT

The effects on growth and flowering of two short-day and two long-day plants when grown under different conditions of illumination are described. The plants fully investigated were *Kalanchoe blossfeldiana* and *Xanthium pennsylvanicum* and the annual varieties of *Hyoscyamus niger* and *Beta vulgaris*. Wintex barley, *Iberis umbellata*, and tomato were also grown in some selected treatments. The conditions investigated comprised continuous full daylight (24 hours), full daylight for the whole of the daily photoperiod and full daylight for half the photoperiods, the other half consisting of either daylight reduced by shading or light from incandescent lamps or fluorescent tubes (daylight-matching type), all of the same low intensity. Two lengths of photoperiod were used for each species, one nearly optimal for flowering, the other closer to the critical day-length; and the order of the low and high light treatments was varied. These factors were combined factorially.

Data were collected (or derived) for the following characteristics, though not always for all the species grown: dry weights, leaf areas, heights, water contents, epidermal cell sizes, net assimilation rates, times to flowering, leaf-number increments until flowering, numbers of inflorescences, stomatal apertures, and leaf postures.

Among other effects, the data revealed that in all four species investigated the adverse effects on over-all growth to be expected from reduction of the daily photoperiod or of the intensity of illumination are in fact minimized. This compensation was effected mainly by large increases in leaf areas, even though in all cases half the daily photoperiod consisted of full daylight. There are indications that increased efficiencies (net assimilation rates) may also have been involved. The leaf-area increases appear to have been due to increased cell size rather than cell number and a close positive correlation with water content was found.

The most striking among the effects on flowering was the failure of sugar-beet to bolt when half of its photoperiod (totals of 20 and 14 hours) consisted of light from fluorescent lamps. The flowering of barley and *Hyoscyamus* was also delayed considerably under these conditions. The deficiency of red in the spectrum of the fluorescent light is believed to have been the cause. By contrast, the flowering of *Iberis*, a crucifer, was not affected.

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INTRODUCTION

IN the study of photoperiodic effects in plants it is frequently necessary to provide conditions of long day or continuous illumination. For this purpose artificial-light sources must be employed since even during midsummer there are only about 16½ hours of daylight in the latitude of southern England. In such experiments the choice lies between the use of artificial illumination throughout or else as a supplement to periods of full daylight. Nearly always the sources of artificial light used differ considerably in their spectral composition from full daylight, and of the two most commonly employed sources, incandescent lamps have an excess of radiation towards the red end of the spectrum, while daylight fluorescent tubes are deficient in this region. Neither source, therefore, is equivalent to ordinary daylight. Of course, it has long been known that artificial light even of low intensity (especially from incandescent lamps) is effective in prolonging the natural day and in evoking the long-day response in plants so treated. None the less, it seemed worth while to compare the effects of artificial-light sources and ordinary daylight.

Apart from the differences in spectral composition it is usually a matter of some difficulty to obtain light intensities comparable with full daylight. Hence a comparative study of artificial-light sources and daylight should also include more than one intensity level of the latter, particularly as the flowering response in plants is markedly affected by low light intensities even if given for only part of the daily photoperiod (cf. also Schwabe, 1954).

Experiments involving such comparisons are possible in the regions north of the Arctic Circle, where the sun remains above the horizon for several weeks during midsummer.

The literature concerned with the effects of light intensity and spectral composition on the growth of plants is very extensive indeed, and only a few papers directly relevant to the experiments to be described below may be cited. One of the first experiments on light effects under Arctic conditions was carried out by Th. Fries (1918) who compared fresh and dry weights, leaf numbers, &c., of several species grown in continuous daylight with those of 12-hour days running either from 8 a.m.–8 p.m. or 8 p.m.–8 a.m. In nearly all instances the largest plants were obtained in continuous daylight (after about 35 days). As expected, of the other two sets the higher-light-intensity set was the larger. Although, with the exception of *Pisum* and *Secale*, the continuous-light plants had higher leaf numbers, Fries pointed out that a greater proportion of assimilate was used for stem growth in those species. N.B. Fries came very near to discovering the photoperiodic response of long-day plants in these experiments. The effects of light intensity under natural outdoor conditions have been investigated in considerable detail by Blackman and Rutter (1948) in relation to the distribution and growth of the bluebell (*Scilla non-scripta*), and Blackman and Wilson (1951 *a*) with regard to several other species. Changes noted when light intensity was reduced include increases in leaf area, reduction of leaf weight per unit area and also proportional reduction in the net assimilation rates. Expressed as g./dm.^{2}/week these rates appeared to be linearly correlated with the logarithm of light intensity.

Pohjakallio (1951) working in Arctic Finland has compared the yields of field crops given different daylengths and receiving different light intensities. He found that the increased daylength in the Arctic does no more than roughly compensate for the decrease in intensity. Incidentally, Pohjakallio and Salonen in an earlier paper (1947) noted an increase in the area of the largest leaflet of potato in a ten-hour day compared with the average natural day of 18 hours 40 minutes, an effect of interest with regard to the results to be presented below. However, no complete analysis of leaf areas was undertaken by the authors. Arthur and Stewart (1935), who compared dry-weight increases over 8 to 11-day periods in continuous light under various artificial-light sources, noted that the relative efficiency of mercury vapour lamps is low compared with Mazda, Neon, or Sodium vapour lights. They concluded that dry-weight production did not appear to be related to the absorption spectrum of chlorophyll.

A series of papers by Roodenburg (1948, 1952) has served to indicate the practical advantages of supplementing winter daylight by using artificial-light sources with or without simultaneous increases in the length of photoperiod.

In experiments carried out by Popp (1926) using daylight from which progressively wider wavebands from the blue part of the spectrum had been filtered out it appeared that the effects on growth made under these conditions of excess red light, &c., resembled those of etiolation, though equal or lower intensities of white light produced no such reactions.

Comparing the effectiveness of light from different spectral regions (used to interrupt a dark period itself following illumination with white light) Borthwick, Parker, and Hendricks (1948) have been able to define action spectra for photoperiodic and other formative responses in plants. Wassink and his co-workers have carried out similar investigations with various species of plants and have noted marked differential effects on flowering behaviour and growth in length as between different spectral regions of visible light, as well as effects due to infra-red radiation (1952 *a*, *b*).

In the present work no attempt was made to distinguish narrow wavebands, indeed this would have been an impossible task, and differential responses noted were due to the aggregate differences between the light sources used.

The work was carried out during the summer of 1952 at Abisko Naturvetenskapliga Station in Swedish Lappland, situated in latitude 68° 20′, and lying about 1,250 ft. above sea-level.

EXPERIMENTAL DESIGN AND METHODS

The object of the experiments was to compare the action of the different light sources and daylight without confounding the effects with any that might arise from gross differences of light intensity. For this reason all plants received full daylight for half their total period of illumination. The effects of light intensity were also investigated by comparing full daylight throughout the photoperiod with conditions of reduced daylight during half the photoperiod. The four qualitative treatments given were: full daylight throughout (D), daylight reduced by shading (butter muslin, occasionally in bright sunlight supplemented by sheets of paper) to the same average intensity as the artificial-light sources employed (S), light from fluorescent tubes (G.E.C. 'colour-matching' type) (F), and light from incandescent lamps (I).

The order of the light treatments was also varied; the artificial-light treatment in some instances preceded the full-light period (*f*) and in others followed it (*a*); in the case of the full-light treatment (D) this represented of course a dummy treatment.

These eight treatments were combined factorially with two daylength conditions, one nearly optimal for flowering and the other near the critical daylength; if necessary this latter daylength was to be altered during the experiment to make it more favourable for flowering and in effect this was done.

Four species of plants were grown under these conditions, two being well known to require short days for flowering (*Kalanchoe blossfeldiana* and *Xanthium pennsylvanicum*), and two species requiring long-day conditions (the annual varieties of *Hyocyamus niger* and *Beta vulgaris*). Six replicates were grown in each treatment. Finally, as an extra treatment, double the number of replicates of each species were grown in full continuous daylight (D 24). The total number of plants in this experiment thus amounted to 432. In addition a few other species were given some selected treatments from the above range. Among these were tomato (var. Carter's (Sunrise)), *Iberis umbellata var. albida*, and 'Wintex' barley. The tomato was chosen for a comparison between the effect of continuous daylight and a day containing some dark period. Crocker (1948) who describes the deleterious effects on the tomato of continuous light consisting wholly or partly of artificial light had also suggested that an experiment with this plant under Arctic daylight conditions might be of interest (p. 304).

Since it was not possible to go to Abisko before the end of May it was necessary to raise the plant material at Rothamsted from seed (*Xanthium*, *Hyoscyamus*, and beet) or from cuttings (*Kalanchoe*). The young plants were grown individually in small cylindrical containers made of waxed paper, the growth medium being a mixture of 75 per cent. Vermiculite and 25 per cent. potting compost. They were watered with a mineral nutrient solution. Paper containers and Vermiculite were chosen because of their light weight, since the plants had to be taken to Sweden by air. During this time, including the travel period and subsequently until the start of the experiment, all plants were kept in daylengths unfavourable for flowering, i.e. long day for *Kalanchoe* and *Xanthium* and short day for *Hyoscyamus* and beet. After arrival in Uppsala the plants were kept for two weeks in a glasshouse of the Institute for Plant Physiology.[1] Finally, they were taken by train (24 hours) to Abisko. In all about 1,000 plants were treated in this way.

The experiment was carried out in an electrically heated greenhouse maintaining a minimum temperature of 15° C. The plants were transferred from their containers to 10-cm. pots into a potting compost; mineral nutrients were supplied several times during the experiment.

The daily light treatments were given by placing the pots to be treated under the muslin shades or fittings for fluorescent or incandescent lights for the requisite times. Complete darkness was achieved by placing large, light-tight cardboard covers (60 × 60 × 75 cm.) over the plants on the greenhouse benches. Sawdust on these prevented possible stray light from reaching the plants under the bottom edges of the boxes.

Ideally, it would have been desirable to arrange the light treatments in such a way that the total number of light quanta absorbed by the plants in treatments (S), (F), and (I) would have been equal in each daylength, in which event any differences would have represented exclusively responses to changes in the spectral composition of the light received, while differences between the responses to (D) and (S) represented intensity effects. No attempt was made to meet such exacting requirements in view of the difficulty of determining the effective absorption spectra for each of the species used, &c.

Instead the much cruder method was used of roughly equilibrating the light sources by means of an ordinary photo-electric cell having a sensitivity curve similar to that of the human eye. Furthermore the shading treatment (S) could not, of course, be maintained at a fixed intensity owing to the diurnal and other fluctuations in the natural illumination. However, by varying the degree of shading several times daily, the average level was made roughly equal to those of (F) and (I). Temperatures under the different lights were equalized by use of compensatory heaters but could not always be made exactly equal to the more fluctuating ambient temperature in the greenhouse (cf. Fig. 1). The final light intensities as measured by the photocell at plant level were about 80–90 foot candles in treatment (I) and 90–100 foot candles in treatment (F). This slightly higher value was chosen because the human eye and hence the photocell used are more sensitive to green light than the average plant, and in contrast to incandescent lamps a higher proportion of the light emitted by the fluorescent tubes used was in this region. The intensity of the natural daylight was determined several times throughout each day and the means per hour have been calculated for the whole experimental period. The graph in Fig. 2 therefore represents the diurnal changes of light intensity during a composite average day.

The daily light régime is shown in Table I; it was altered after about 5 weeks by shortening or lengthening the light periods for the S.D. and L.D.

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[1] The author is indebted to Professor E. Melin for this hospitality.

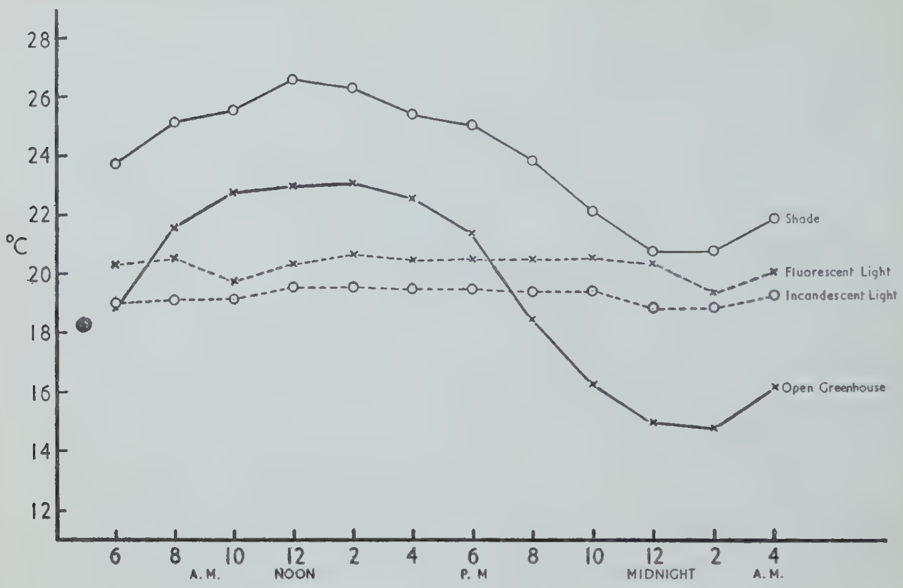

FIG. 1. Mean temperature throughout 24 hours.

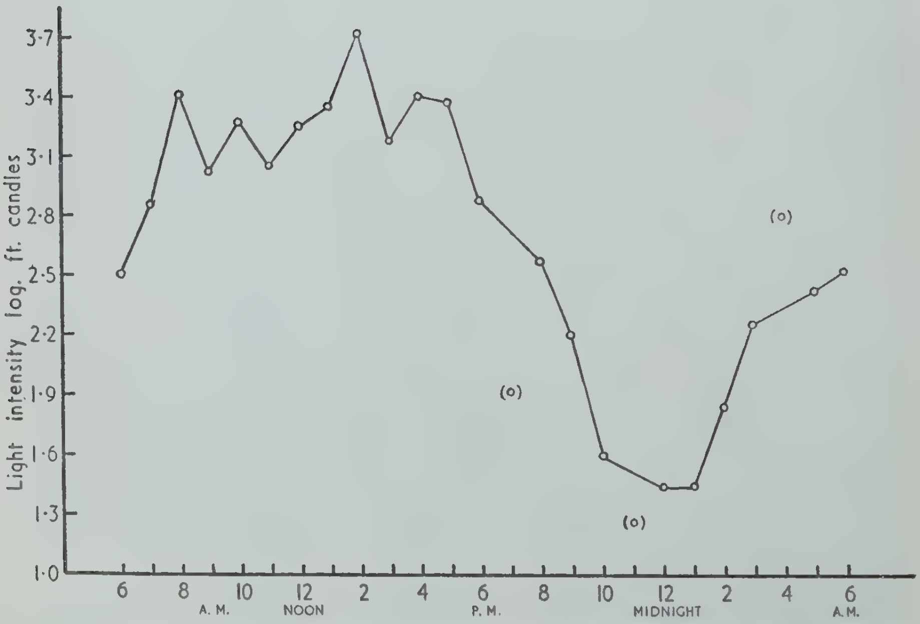

FIG. 2. Average intensity of daylight throughout 24 hours (log. ft. candles). (Points in brackets are means of 2 or 3 determinations only.)

plants respectively by 2 hours in order to make those near the critical daylengths more favourable for inducing speedier flowering.

TABLE I

Régime of Light Treatments

Species	Treatment	Dark		Full daylight		Artificial or reduced light	
		from	to	from	to	from	to
Xanthium *Kalanchoe*	D 8f	4 p.m.	8 a.m.	8 a.m.	4 p.m.	—	—
	D 8a	4 p.m.	8 a.m.	8 a.m.	4 p.m.	—	—
	S, F, I 8f	4 p.m.	8 a.m.	12 noon	4 p.m.	8 a.m.	12 noon
	S, F, I 8a	4 p.m.	8 a.m.	8 a.m.	12 noon	12 noon	4 p.m.
Xanthium *Hyoscyamus* Beet Wintex barley *Iberis* (from 8/7/52)	D 14f	10 p.m.	8 a.m.	8 a.m.	10 p.m.	—	—
	D 14a	10 p.m.	8 a.m.	8 a.m.	10 p.m.	—	—
	S, F, I 14f	10 p.m.	8 a.m.	3 p.m.	10 p.m.	8 a.m.	3 p.m.
	S, F, I 14a	10 p.m.	8 a.m.	8 a.m.	3 p.m.	3 p.m.	10 p.m.
Kalanchoe (from 8/7/52)	D 10f	6 p.m.	8 a.m.	8 a.m.	6 p.m.	—	—
	D 10a	6 p.m.	8 a.m.	8 a.m.	6 p.m.	—	—
	S, F, I 10f	6 p.m.	8 a.m.	1 p.m.	6 p.m.	8 a.m.	1 p.m.
	S, F, I 10a	6 p.m.	8 a.m.	8 a.m.	1 p.m.	1 p.m.	6 p.m.
Hyoscyamus Beet Wintex barley *Iberis*	D 20f	10 a.m.	2 p.m.	2 p.m.	10 a.m.	—	—
	D 20a	10 a.m.	2 p.m.	2 p.m.	10 a.m.	—	—
	S, F, I 20f	10 a.m.	2 p.m.	12 mdnt.	10 a.m.	2 p.m.	12 mdnt.
	S, F, I 20a	10 a.m.	2 p.m.	2 p.m.	12 mdnt.	12 mdnt.	10 a.m.
Xanthium *Kalanchoe* *Hyoscyamus* Beet Wintex barley *Iberis*	D 24			throughout 24 hrs.			
				From 7/6/52 to 8/7/52			
Kalanchoe *Hyoscyamus* Beet Wintex barley *Iberis*	D 12f	8 p.m.	8 a.m.	8 a.m.	8 p.m.	—	—
	D 12a	8 p.m.	8 a.m.	8 a.m.	8 p.m.	—	—
	S, F, I 12f	8 p.m.	8 a.m.	2 p.m.	8 p.m.	8 a.m.	2 p.m.
	S, F, I 12a	8 p.m.	8 a.m.	8 a.m.	2 p.m.	2 p.m.	8 p.m.
Xanthium	D 16f	4 p.m.	12 mdnt.	12 mdnt.	4 p.m.	—	—
	D 16a	12 mdnt.	8 a.m.	8 a.m.	12 mdnt.	—	—
	S, F, I 16f	4 p.m.	12 mdnt.	8 a.m.	4 p.m.	12 mdnt.	8 a.m.
	S, F, I 16a	12 mdnt.	8 a.m.	8 a.m.	4 p.m.	4 p.m.	12 mdnt.

DATA COLLECTED

The data derived from the experiment fall into two classes, those measuring the treatment effects on the growth of the plants and those assessing the flowering response. It was not always possible to collect the same data for all the species used nor for all the six replicates in some cases. Furthermore the two daylength treatments were sampled at different times so that for some comparisons daylength effects on growth are confounded with sampling dates. The information lost in this way was not regarded as very important and in any case it would have been misleading to have maintained one set of plants after budding (i.e. during a period when no new leaves would have been produced) for comparison with another still growing vegetatively.

A preliminary sample recording the vegetative characteristics of the plants at the start of the experiment was also taken. In the case of *Xanthium* and beet the number of expanded and expanding leaves was also noted at this time as well as their node numbers above the cotyledons. From these data it was later possible to estimate the area of these leaves, making allowance for the fact that the youngest leaves present at that time were only partly expanded. In the leaf-area determinations carried out at the time of sampling the outline of each leaf was drawn on paper and the area subsequently measured with a planimeter. Knowing the node number corresponding to each measured leaf it was possible to estimate the effects of treatments on the area of single leaves of different ages in their order of production. Also, using the data of the preliminary sample it was possible to calculate the Net Assimilation Rates for one long-day and one short-day plant (*Xanthium* and beet). Owing to the comparatively long interval between the preliminary and subsequent samples, the objections of Williams (1946) to the usual method of calculating N.A.R. (here employed also) cannot be disregarded. However, the absolute values of the N.A.R.s obtained are of little importance; the rate being required chiefly for comparisons between treatments. The values have been expressed in two ways: (1) in the form most commonly employed, i.e. as mg./cm^2./week, and (2) making allowance for the period of illumination as mg. CO_2/dm^2./hours full light plus 20 per cent. of the period in low light, i.e. assuming all dry-weight increases to be in the form of hexose sugars. Where the daylength was altered (in one series in *Xanthium* from 16 hours to 14 hours and in one in beet from 12 hours to 14 hours) the appropriate adjustment was made.

In *Xanthium* it was noted that the 'posture' of the leaves differed in the various treatments and that particularly in some treatments there were signs of epinasty. The degree of epinasty was therefore estimated on one occasion for about half the number of treatments in the following manner: assuming the leaf curvature from the base of the petiole to the tip of the blade to form part of a circle, which is approximately true, it was relatively easy to measure the chord of the segment formed by the leaf on this circle, ($2a$) in the diagram (Fig. 3). The horizontal distance of the tip of the leaf to the upright stem ($2b$) was also measured and thus it was possible to obtain the sine of the angle (α) between the stem and the tangent to the centre of the leaf (b/a), this being a composite measure of the general curvature of the leaf and the angle of the petiole with the stem. Thirdly, measurement of the total length of the leaf ($2c$) yielded an estimate of the angle subtended by the leaf at the centre of the circle. Making use of these two angular values, the angle of the petiole with the stem at its point of insertion could also be calculated.

The angle (2γ) subtended by the leaf at the centre of the circle of which it formed an arc was determined as follows from the known lengths $2c$ and $2a$: $\gamma = c/r$, $\sin\gamma = a/r$, $\gamma/\sin\gamma = c/a$. Plotting the ratio $\sin\gamma/\gamma$ against 2γ, the observed values for the ratio a/c could then be found on this graph reading off the angle subtended by the leaf at the centre of the circle passing through its midrib and petiole. As can be easily shown the angle (β) between the petiole and the stem at its point of insertion (tangent to this point) is then found by subtracting α and γ from 180. These values and those for the over-all curvature of the leaves are presented.

Estimates of stomatal apertures at the end of the different light and dark periods were made from Lloyd's strips; they are presented for *Hyoscyamus niger* and *Xanthium pennsylvanicum*, expressed as mean diameters of the stomatal apertures in μ.

Finally in the 14-hour photoperiod series of beet rectangular pieces of leaf blade approximately half-way along the length of the leaf were cut out from 3 replicate plants in each treatment and preserved in alcohol. Subsequently estimates of cell size were made for both upper and lower epidermes by measuring the areas of groups of 10 cells by planimeter from camera lucida drawings. Stomatal guard cells (about 3 per cent. of the area) were omitted from these. From each leaf strip 5 sample areas were measured. The mean area per cell converted to μ^2 is presented in Table XV.

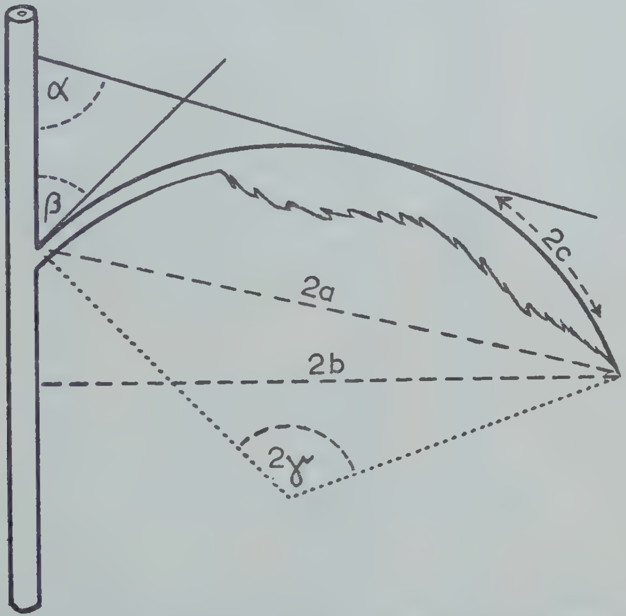

FIG. 3. Method of determining angle of insertion of petiole (β) and general leaf curvature (angle 2γ subtended by leaf at the centre of the circle of which petiole and midrib form an arc). Cf. text.

Where possible the data have been analysed statistically and only significant effects will be discussed. In some cases transformation of the original data was required and here the transformed values are presented.

RESULTS

Before introducing the numerical results a few words may be said about the general appearance of the plants in the different light treatments. As little as ten days after the start of differential treatment those plants receiving full daylight for 24 hours became noticeably different in appearance. This was due mainly to the large amounts of red pigment, probably anthocyanin, being produced in them. *Xanthium* and annual beet were the most obviously affected; *Kalanchoe* was rather less so, though even in this species the edges of the leaves became bright red. Petioles and stems of *Xanthium* were almost purple, those of beet a bright red. Although the appearance of the continuous-daylight plants resembled that of plants suffering from nitrogen deficiency—a high rate of photosynthesis might conceivably have led to internal N-deficiency—this was not relieved by external application of nutrients. A little later effects on leaf areas and heights of plant became obvious; the magnitude of these effects may be seen from the measurements presented below.

TABLE IIA

Xanthium. Treatment Means

	Total dry wt. (g.)	Dry wt. tops (g.)	Water cont. tops (% dry wt.)	Height incr. at budding (cm.)	sq. rts.*	Days to flowering	Leaf No. incr. to flowering
D 24 (2)	2·681	1·610	321	[19·5]†	—	—	[7·5]†
D 14f	2·425	1·423	383	3·9‡	2·38	34·5‡	6·0‡
a	2·292	1·352	417	8·0		43·0	8·2
S 14f	2·030	1·280	453	9·3	2·98	41·7	8·2
a	2·238	1·407	413	8·6		43·0	8·2
F 14f	1·966	1·236	500	9·1	2·91	42·0	8·2
a	2·351	1·509	421	8·3		41·8	8·3
I 14f	1·962	1·373	545	21·4	4·38	43·2	8·2
a	2·381	1·728	398	17·4		42·3	8·2
D 24 (1)	1·729	0·929	315	[20·3]†	—	—	[4·5]†
D 8f	1·436	0·874	397	1·3	1·11	16·2	4·3
a	1·402	0·861	387	1·3		16·6	4·5
S 8f	1·234	0·788	419	1·6	1·25	16·2	4·5
a	1·225	0·794	448	1·7		15·6	4·9
F 8f	1·215	0·750	437	1·0	1·02	16·3	4·2
a	1·098	0·701	443	1·1		16·0	4·2
I 8f	1·270	0·815	436	4·0	1·90	16·3	4·3
a	1·251	0·813	479	3·3		15·8	4·5

* Square root transformation of original data.
† Vegetative plants.
‡ Due to treatment error.

The numerical results are presented in Tables II to VIII. Statistically significant comparisons are shown in Tables IX to XII, from which, however, some very obvious effects such as increases in dry weight due to differences in sampling time, &c., have been omitted.

Effects of continuous daylight

In the two short-day species *Xanthium* and *Kalanchoe* the heaviest plants were produced under conditions of continuous daylight—plants which remained of course quite vegetative. In the two long-day species, on the other hand, plants in continuous daylight were not significantly larger than those receiving some darkness, and in the tomato, higher dry weights were realized in a 16-hour day than in continuous light, though the effect failed to reach

TABLE IIB

Xanthium. Treatment Means (cont.)

	Total leaf area (per plant) cm^2.	Leaf area per leaf No.						N.A.R. as:	
		5	6	7	8	9	10	mg./cm.^{2}/ week	mg. CO_2/dm.^{2} hours full light+20%*
D 24 (2)	131·4	—	17·1	16·6	19·6	22·4	24·0	5·25	4·58
D 14f	150·0	12·5	16·0	18·0	22·6	25·8	26·4	4·02	5·43
a	165·5							3·48	4·70
S 14f	179·5	14·6	15·4	18·0	22·0	25·0	28·6	2·78	6·25
a	184·9	10·3	18·3	20·7	26·8	31·8	31·6	3·13	7·05
F 14f	197·3	13·3	16·4	17·8	22·2	28·2	35·5	2·45	5·52
a	192·7	14·8	17·9	21·0	28·1	31·5	32·6	3·27	7·35
I 14f	190·1	11·8	16·2	18·2	27·2	32·2	37·3	2·50	5·63
a	194·5	13·0	17·1	21·7	26·8	33·9	35·0	3·32	7·47
D 24 (1)	70·2	12·4	16·2	15·3	13·8	8·0	1·4	5·73	5·00
D 8f	107·4	14·0	18·1	19·0	17·7	12·0	8·9	3·13	8·20
a	102·2							3·06	8·02
S 8f	118·7	12·7	16·2	20·3	21·0	18·4	13·0	2·08	9·08
a	120·1	12·8	17·6	22·3	22·3	16·2	10·6	2·03	8·87
F 8f	118·5	14·1	18·8	21·3	19·2	13·8	8·8	2·00	8·73
a	120·7	14·8	18·0	21·4	21·9	14·1	8·7	1·49	6·50
I 8f	126·5	13·5	18·6	22·8	20·0	15·4	11·2	2·15	9·38
a	141·8	15·8	19·0	25·9	23·5	16·8	11·6	1·93	8·43

* Except, of course, treatments D and D 24.

TABLE III

Kalanchoe. Treatment Means

Treatment	Total dry wt. (g.)	Water content (% dry wt.)	Height increment (cm.)	Days to flowering	No. of inflorescence axes
D 24 (2)	3·90	816	[10·5]*	—	—
D 10f	2·54	1,262	5·3	44·3	24·0
a	3·02	1,200	5·1	45·0	26·0
S 10f	1·85	1,412	8·0	44·7	17·3
a	2·45	1,364	5·8	45·2	28·7
F 10f	1·64	1,503	5·9	44·3	19·0
a	2·21	1,419	5·6	45·8	21·7
I 10f	1·82	1,360	7·5	45·5	17·3
a	2·26	1,241	6·9	46·5	19·0
D 24 (1)	1·98	929	[5·4]*	—	—
D 8f	1·38	1,643	1·4	19·8	17·3
a	1·27	1,761	1·2	20·2	14·7
S 8f	0·96	1,986	1·8	20·7	13·3
a	1·17	1,799	2·2	20·7	13·0
F 8f	1·01	1,680	1·5	21·0	14·0
a	1·05	1,680	1·2	21·8	7·3
I 8f	0·71	2,183	1·8	21·3	12·0
a	0·92	1,826	1·6	21·0	13·7

* Vegetative plants.

TABLE IV

Annual Beet. Treatment Means

	Dry wt. tops and tap root. (g.)	Water content tops and tap root. (% dry wt.)	Total Leaf area (per plant) (cm.^{2})	N.A.R. as:		Days to shooting	Days to anthesis	Leaf No. increment to flowering
				mg./cm.^{2}/ week	mg.CO$_2$/dm.^{2}/ hours full light +20%*			
D 24	1·045	471	61·4	3·53	3·08	25·5	44·5	10·1
D 20f	1·096	548	107·9	2·65	2·78	28·1	50·7	11·0
a	1·070	666	112·1	2·52	2·64	29·2	52·0	11·3
S 20f	1·005	689	136·1	2·08	3·63	26·7	45·8	11·7
a	0·874	724	133·9	1·81	3·17	27·7	44·7	10·5
F 20f	0·886	892	166·8	1·59	2·78	50·0 (2 pl.)	—	19·5
a	0·615	946	138·5	1·20	2·10	—	—	(—)
I 20f	0·921	664	120·4	2·05	3·58	25·2	43·3	9·1
a	0·980	757	161·1	1·82	3·18	27·2	46·8	11·5
D 14f	1·016	621	124·9	1·87	3·01	—	—	(18·0)†
a	1·172	551	118·6	2·25	3·63	—	—	(15·3)
S 14f	1·274	619	191·8	1·81	4·87	65 (1 pl.)	—	(18·5)
a	1·527	564	180·6	2·28	6·13	65 (1 pl.)	—	(16·8)
F 14f	0·966	771	182·8	1·39	3·73	—	—	(17·8)
a	1·399	628	185·6	2·04	5·48	—	—	(17·7)
I 14f	1·336	696	251·6	1·59	4·27	—	—	(18·1)
a	1·585	555	191·8	2·28	6·13	65 (1 pl.)	—	(19·1)

* Except, of course, treatments D and D 24.

† Vegetative plants at sampling time.

TABLE V

Hyoscyamus niger. Treatment Means

	Dry wt. tops (g.)	Water content tops (% dry wt.)	Total leaf area (per plant) (cm.^{2})	Days to shooting	Days to anthesis	Leaf No. increment to 1st flower
D 24	0·498	520	72·2	20·7	36·5	12·7
D 20f	0·381	752	93·6	21·0	39·2	12·7
a	0·480	705	107·4	20·5	39·2	12·7
S 20f	0·381	706	101·0	20·3	37·5	12·7
a	0·341	720	94·6	18·7	35·3	11·8
F 20f	0·445	811	132·7	21·8	40·8	13·8
a	0·409	869	165·6	23·0	43·5	12·8
I 20f	0·489	616	102·0	17·5	35·0	12·5
a	0·388	721	111·2	18·2	38·9	12·7
D 14f	—	—	—	42·7	63·5*	21·8
a	—	—	—	42·1	62·3*	21·8
S 14f	—	—	—	39·1	62·8*	20·2
a	—	—	—	44·3	> 67·0*	23·8
F 14f	—	—	—	41·2	> 67·0*	21·0
a	—	—	—	48·7	> 67·0*	25·5
I 14f	—	—	—	36·2	64·4*	19·6
a	—	—	—	41·5	60·0*	21·0

* Value of 67 days put in where flower had not yet opened at the end of the experiment.

statistical significance: the fresh weights of these plants were definitely above those in continuous light, their water content being higher. In *Xanthium*, *Kalanchoe*, and sugar-beet also, very much reduced water contents were found in the absence of darkness, all other daylength and light treatments showing greater succulence.

TABLE VI

Tomato. Treatment Means

	16 hours' daylight	24 hours' daylight	Significance of 't' test
Dry weight tops (g.)	2·836	2·583	P = <0·2 not sig.
Water content (% dry weight)	510	438	P = <0·01
Height (cm.)	36·5	28·4	P = <0·001
Leaf area (cm.^{2})	274·2	207·6	P = <0·02
Leaf number at end of experiment	11·2	10·2	Not analysed

TABLE VII

Wintex Barley. Treatment Means

Treatment	Days to anthesis	Final leaf No.	Height on: *8.8.52* (cm.)	Days to awn emergence
D 24	37·5	6·25	34·0	31·0
D 20	46·5	7·25	34·5	38·0
S 20	47·3	7·00	33·6	38·5
F 20	> 69	9·25	33·5	64·5
I 20	41·0	6·50	31·6	33·5
			23.7.52	
D 14	64·0*	9·50	38·0	55·5
S 14	> 69	10·50	33·6	—
F 14	> 69	10·25	6·6	—
I 14	> 69	9·25	36·8	64·8
Sig. difference	2·84†	0·89	—	—

* Including 1 plant assumed to have reached anthesis after 70 days.

† This applies only to treatments D 24, D 20, F 20, I 20.

TABLE VIII

Iberis. Treatment Means

Treatment	Leaf No. to flower	Days to budding	Height at budding
D 24	10·3 (2·1)*	29·2	7·9 cm.
D 20	9·4 (2·2)	31·4	9·2 cm.
S 20	8·0 (2·4)	28·8	9·0 cm.
F 20	7·8 (2·4)	31·2	9·4 cm.
I 20	7·9 (2·3)	29·8	9·6 cm.
Sig. difference	1·16	2·58 not sig.	—

* No. of bracts in parentheses.

Total leaf areas in *Xanthium*, *Hyoscyamus*, and beet were much the smallest under this treatment, amounting in the first two species to only 67 per cent. and 64 per cent. of the average of those treatments having some darkness; in sugar-beet the reduction was still greater, e.g. to 45·5 per cent. even when the comparison is confined to the longer (20-hour) daylength treatments in which all plants flowered. In the tomato, leaf area was reduced to 75·7 per cent. of that of the plants receiving 16 hours' full daylight. Reinders-Gouwentak *et al.* (1951) also noted decreased leaf areas in tomatoes grown entirely in artificial light when the daylengths were extended by low-intensity light.

TABLE IX. *Xanthium. Significant Effects*

Characteristic	Effect	General mean	Treatments compared (i)	Treatments compared (ii)	Effect i.e. difference (ii)—(i)	Sig. difference
Total dry weight	Duration of light	1·788 g.	D 8	D 24	0·310	0·257
			D 14	D 24	0·322	
	Light intensity		S	D	0·207	0·148
	Order of low-light treatment		f 14	a 14	0·220	0·148
Dry weight of tops	Duration of light	1·124 g.	D 14	D 24	0·222	0·174
	Spectral composition		S	I	0·115	0·100
			F	I	0·133	
	Order of low-light treatment		f 14	a 14	0·171	0·100
Water content of tops	Duration of light	387·5% of dry weight	D 24	D 8	77·0	59·4
			D 24	D 14	79·0	
	Light intensity		D	S	37·0	25·2
	Spectral composition		S	F	17·0	25·2
			S	I	31·0	
	Order of low-light treatment		a 14	f 14	57·8	25·2
Total leaf area per plant	Duration of light	145·1 $cm.^2$	D 24	D 8	34·6	15·1
			D 24	D 14	26·4	
	Light intensity		D	S	19·6	8·8
	Spectral composition		S	F	6·5	8·8
			S	I	12·4	
Leaf area per leaf:						
No. 6	Duration of light	17·3 $cm.^2$	14	8	1·3	1·26
No. 7	,,	19·9 $cm.^2$	14	8	2·3	1·60
	,,		D 24	D 8	3·7	3·89
	Light intensity and spectral composition		D	S = F	1·8	2·26
			D	I	3·7	
	Order of low-light treatment		f	a	2·4	1·60
No. 8	Duration of light	21·94 $cm.^2$	D 24 (1)	D 8	3·9	4·28
			D 24 (2)	D 14	3·0	
	Light intensity		D	S	2·8	2·48
	Order of low-light treatment		f	a	3·0	1·76
No. 9	Duration of light	21·3 $cm.^2$	D 24 (1)	D 8	4·0	4·03
			D 24 (2)	D 14	3·4	
	Light intensity		D	S	3·9	2·32
	Spectral composition		F	S	0·9	2·32
			F	I	2·7	
No. 10	Light intensity	20·03 $cm.^2$	D	S	3·3	2·54
	Spectral composition		S = F	I	2·8	2·54
Square root of height incr. at budding	Light intensity	2·24 $\sqrt{cm.^2}$	D 8	S 8	0·15	0·175
			D 14	S 14	0·60	
	Spectral composition		F 8	S 8	0·24	0·175
			S 8	I 8	0·64	
			F 14	S 14	0·07	
			S 14	I 14	1·40	

* Omitting effects due to age and sampling time.

In the analysis of the areas of individual leaves a significant difference due to daylength is found as early as leaf No. 6. This leaf had already begun to expand at the start of differential treatment. The shorter photoperiod or perhaps more probably the longer dark period of the 8-hour day led to the increase in leaf area compared with the 14-hour day. This effect was maintained for the next leaf (No. 7, already separated from the bud at the start of the experiment) but thereafter it was reversed owing to the earlier flowering and consequent sampling of the D 8 series. Beginning with leaf No. 7 continuous light (D 24) led to reduced leaf area, relative to the mean of the other light treatments. This effect is almost statistically significant even when the comparison is confined to the full daylight treatments, as it is in Table IX. The difference was maintained for leaves 8, 9, and 10 even after excluding the set of D 24 plants which were harvested simultaneously with all 8-hour photoperiod treatments, whose inclusion would have artificially exaggerated the effect. The differences in the areas of leaves Nos. 8, 9, and 10 between the two daylengths 8 and 14 hours are of no interest since in the main they merely reflect differences due to age at sampling time.

TABLE X

Kalanchoe. Significant Effects

Characteristic	Effect	General mean	Treatments compared		Effect i.e. difference (ii)—(i)	Sig. difference
			(i)	(ii)		
Dry weight of tops	Duration of light	1·16 g.*	D 8	D 24	0·66	0·281
		2·41 g.†	D 10	D 24	1·12	0·418
	Light intensity	1·16 g.	S 8	D 8	0·25	0·229
	,,	2·41 g.	S 10	D 10	0·63	0·340
	Spectral composition	1·16 g.*	F 8	S 8	0·04	0·229
			I 8	S 8	0·25	
		2·41 g.†	F 10	S 10	0·23	0·340
			I 10	S 10	0·11	
	Order of low-light treatment	2·41 g.†	f 10	a 10	0·53	0·240
Water content	Duration of light	1,820 % of dry wt.*	D 24	D 8	773	380
		1,345 % of dry wt.†	D 24	D 10	415	87
			D 10	D 8	471	87
	Light intensity	1,345 %†	D 10	S 10	157	71
	Spectral composition	,,	S 10	F 10	73	71
			I 10	S 10	87	71
	Order of low-light treatment	,,	a 10	f 10	78	71
Height increment to budding	Light intensity	3·91 cm.	D	S	1·21	0·49
	Spectral composition	,,	F	S = I	0·90	0·49
	Order of low-light treatment	,,	a	f	0·71	0·34
Number of inflorescence axes	Light intensity	13·2	S 8	D 8	2·8	2·13
	Spectral composition	,,	F 8	S 8	2·5	2·13
			F 8	I 8	2·1	
	Order of low-light treatment	21·6	f 10	a 10	4·4	1·5

* First sample, photoperiods of 24 and 8 hours.
† Second sample, photoperiods of 24 and 12 hours.

Plant heights in the tomato were also much reduced by continuous daylight. No significant effects are to be seen in the heights at anthesis in beet, henbane, and barley, while no valid comparisons can be made in the two short-day species between the height increments of the vegetative plants in D 24 and those noted at the time of budding in all other treatments.

TABLE XI

Annual Beet. Significant Effects

Characteristic	Effect	General	Treatments compared (i)	Treatments compared (ii)	Effect i.e. difference (ii)—(i)	Sig. difference
Dry weights of tops + tap root	Light intensity	0·954 g.*	S 20	D 20	0·143	0·148
		1·285 g.†	D 14	S 14	0·307	0·215
	Spectral composition	0·954 g.*	F 20	S 20	0·189	0·148
			S 20	I 20	0·010	
		1·285 g.†	F 14	S 14	0·218	0·215
			S 14	I 14	0·060	
	Order of low-light treatment	1·285†	f 14	a 14	0·273	0·152
Water content	Duration of light	683% of dry wt.*	D 24	D 20	135	79·7
	Light intensity	"	D 20	S 20	99	79·7
	Spectral composition	"	S 20	F 20	213	79·7
		"	I 20	F 20	208	
		626% of dry wt.*	S 14	F 14	109	65·1
			I 14	F 14	74	
	Order of low-light treatment	683%*	f 20	a 20	84	56·3
		626%†	a 14	f 14	102	46·0
Leaf area per plant	Duration of light	146·0 cm.^{2}	D 24	D 20	58·6	22·1
	Light intensity		D 20	S 20	25·0	18·0
			D 14	S 14	64·4	
	Spectral composition		S 20	F 20	17·6	18·0
			S 20	I 20	5·8	
			S 14	I 14	35·5	
			F 14	I 14	37·5	
	Order of low-light treatment		a 14	f 14	22·7	12·8
Days to shooting	Duration of light	26·9 days	D 24	D 20	3·2	1·9
	Light intensity		S 20	D 20	1·5	1·9
	Spectral composition		I 20	S 20	1·0	1·9
			S 20	F 20	> 25	
Cell size upper epidermis (area of external wall)	Light intensity	2,230 μ^2/cell	D 14	S 14	362	934
	Spectral composition		S 14	F 14	807	934
			S 14	I 14	1,664	
Cell size lower epidermis (area of external wall)	Light intensity	1,484 μ^2/cell	D 14	S 14	245	658
	Spectral composition		S 14	F 14	385	658
			S 14	I 14	1,190	

* First sample, photoperiods of 24 and 20 hours.
† Second sample, 14-hour photoperiod treatments.

The derived net assimilation rates of *Xanthium* seem to be of normal magnitude under conditions of uninterrupted illumination (cf. Heath and Gregory, 1938), if expressed in the usual manner as mg./cm.^{2}/week. The N.A.R.s for sugar-beet are rather lower, but the mean value for a 20-hour day (2·59 mg./cm.^{2}/week) does not differ much from values obtained by Watson and Watson (1953) (mean of their three control sets 2·87 mg./cm.^{2}/week). When the differences in the periods of illumination are taken into account (N.A.R.s expressed as mg.CO_2/dm.^{2}/hour light), continuous light appears to result in a lower average efficiency than is found in treatments having a daily dark period. Again the reactions of *Xanthium* and beet do not show marked differences in spite of the difference in their daylength responses.

Flowering responses were, of course, confined to the long-day species. In *Hyoscyamus*, *Beta*, and also *Iberis*, no differences appeared between the D 24 and D 20 treatments; if anything D 24 led to more rapid flowering. However, in *Hyocyamus* at least this did not represent the optimum treatment for I 20 flowered significantly sooner (see below). In barley, on the other hand, continuous light hastened anthesis by nearly 9 days and the final leaf number was reduced by 1·0.

Finally, in *Xanthium* this treatment resulted in a greater degree of epinasty than did any other.

TABLE XII

Hyoscyamus. Significant Effects

Characteristic	Effect	General mean	Treatments compared		Effect i.e. difference	Sig. difference.
			(i)	(ii)	(ii)—(i)	
Leaf area per plant	Duration of light	108·9 $cm.^2$	D 24	D 20	28·3	14·16
	Spectral composition		S	F	51·4	11·56
			I	F	42·6	
	Order of low-light treatment		f	a	12·4	8·18
Days to shooting	Duration of light	29·90 days	D 24	D 20	0·1	1·6
			D 20	D 14	21·6	
	Light intensity		S	D	1·1	0·8
	Spectral composition		S	F	3·1	0·8
			I	S	2·3	
	Order of low-light treatment		f 14	a 14	4·38	0·8

Light-intensity effects

Treatments D and S differed only in the intensity of the daylight received during the photoperiod. As was to be expected total dry weights were diminished in the S treatments. The difference is significant in the two short-day species in both daylengths, but in beet the effect is confined to the longer (20 hours) daylength.

Water contents varied almost inversely with the dry weights and shading increased the succulence of *Xanthium*, *Kalanchoe*, and beet, though in the latter two species the effect is significant only in the longer daylength.

Leaf areas in *Xanthium* and beet were considerably larger when half the photoperiod was of reduced intensity, this being so in both daylengths. The greater dry weight in the shaded beet plants with a 14-hour photoperiod was probably due to the much increased leaf area in this treatment. The effect of shade in increasing leaf area became apparent in *Xanthium* in the last leaf to have separated from the bud at the beginning of the experiment (No. 7), and thereafter was maintained for all subsequent leaves whose areas were analysed individually. There also appears to have been a slight interaction with the length of day, shading being more effective in the shorter daylength.

In *Xanthium* and *Kalanchoe* a significant increase in height of plant at the time of budding was also noted in the S treatments, relative to that achieved under full daylight throughout the photoperiod.

As might be expected the N.A.R.s of *Xanthium* and beet were reduced by shading but if allowance is made for the period of reduced light (e.g. by assuming the reduction of the intensity which was to a maximum $\frac{1}{5}$ full daylight, to be equivalent to reduction of duration to $\frac{1}{5}$ of the time actually spent in reduced light) it is found that the N.A.R.s of the shaded plants were really as high or higher than those of the D series. The effects of reduced light on total photosynthesis are therefore apparently compensated by greater efficiency as well as by increased leaf areas.

The flowering response was not much affected by shading in the case of *Xanthium*, but in *Kalanchoe* the number of inflorescence axes was significantly higher in full daylight throughout an 8-hour day, the shading treatment amounting to about 82 per cent. of the full daylight set. In barley also anthesis appears not to have been affected by light intensity.

In the two long-day species, beet and *Hyoscyamus*, there were, however, clear effects of shading on flowering. In both these species as also in *Iberis* there was a definite, though slight, acceleration due to shading, which is in good agreement with effects noted by Samygin (1948). There was, however, a strong interaction with the order in which the full light and shading periods were presented, the earliest flowering occurring when the reduced-light preceded the full-daylight period.

Effects due to the quality of light

Differences in light quality are involved in the comparisons between treatments S, F, and I. As was pointed out above, however, the differences caused by the use of these three light sources during the reduced-light part of the photoperiod are not entirely due to differences in spectral composition, for some differences in the total energies absorbed by the plants must also be confounded with purely spectral effects.

As far as total dry weights are concerned incandescent light generally produced plants which were as heavy as those in reduced daylight; only in *Kalanchoe* in 8-hour days was there a slight reduction. Fluorescent light during half the photoperiod led to significant reductions in total dry weight in beet and to almost significant reductions in *Kalanchoe* in the longer daylength; but in *Xanthium*, *Hyoscyamus*, and *Kalanchoe* on 8-hour days there were no significant differences.

Leaf areas in *Xanthium* fell into the following order of increasing magnitude: $S < F < I$, the difference between the extremes being statistically significant. In the annual beet the order was identical, but in *Hyoscyamus* it was $S < I < F$. In *Xanthium* the effect of light quality was seen first in leaf No. 7 and the effect remained unaltered for the next three leaves to be produced. While fluorescent lamps and reduced daylight did not differ significantly in their effects, incandescent light led to markedly increased leaf areas in both daylengths given.

The height increments of the plants from the start of the experiments until macroscopic budding fell into the following order for *Xanthium*: $F = S < I$ (analysed on the square roots of the original values). The actual mean increase of treatment I exceeded those of F and S by 100 per cent. In *Kalanchoe* the order was $F < S = I$, only very little growth having been made under fluorescent lighting.

The N.A.R.s do not seem to have been much affected by the spectral composition of the low-intensity part of the daily illumination, being perhaps somewhat reduced in beet receiving fluorescent lighting.

The flowering responses of *Xanthium* appear to have been unaffected by the differences in light source during the low-light period, but in *Kalanchoe* the numbers of inflorescence axes in the 8-hour day were in the order: $F < I = S$, the difference being significant. In the longer daylength also treatment S had the highest average but this difference was not significant.

In the long-day species, however, quite large effects were found due to light source. In the case of beet there was little difference between treatments S and I, but treatment F (fluorescent lights) resulted in almost complete failure to flower even when the total photoperiod was as much as 20 hours a day. In *Hyoscyamus* also fluorescent lighting caused a delay in shooting of several days. Incandescent light had the opposite effect, flowering being significantly accelerated. In fact, under a 20-hour day shooting occurred sooner in this treatment than in any other including even continuous daylight.

In barley also anthesis occurred with incandescent light even earlier than in a 20-hour day of full daylight throughout, and the leaf number was almost as low as that found in continuous light. Fluorescent light for half the 20 hours' photoperiod, on the other hand, delayed anthesis by nearly 4 weeks and in a 14-hour day the plants had only just begun to elongate when the experiment had to be ended. Moreover in the 20-hour day the leaf-number of the main shoot before earing was increased by 3 under this illumination.

The cruciferous long-day plant *Iberis* behaved quite differently since it flowered in the fluorescent-light treatment with the lowest leaf number of all treatments.

Effects due to the sequence of low- and high-light periods

Higher total dry weights were found in *Xanthium*, *Kalanchoe*, and beet when the photoperiod began with full daylight than under those treatments in which the reduced light followed the dark period. In the two short-day species the effect was confined to the longer daylength, in sugar-beet to the shorter (12 hours).

Water contents in *Xanthium*, *Kalanchoe*, and beet were affected in the opposite direction to dry weights. In beet exposed to the 20 hours' day, however, the reverse situation was obtained and water contents were lower when the low light preceded daylight.

Among the various characteristics measured leaf areas and plant heights were affected only to a slight extent by the sequence of high and low light. In

Xanthium the areas of leaves Nos. 7, 8, and 9 were somewhat larger in those treatments in which the first half of the photoperiod consisted of full daylight than in the 'low light first' set. The effect was no longer apparent in the youngest leaf analysed. N.A.R.s appeared somewhat higher when full light commenced the photoperiod, in *Xanthium* under a 14-hour day, in beet under one of 12 hours.

However, the only really highly significant effect associated with the sequence of the light treatments was on the flowering of *Hyoscyamus* and to a lesser extent of beet. In each case the time to shooting was reduced in those treatments receiving low daylight or artificial light first in the photoperiod. The effect was particularly marked in *Hyoscyamus* in the less favourable day-length (12 hours).

Effects on stomatal apertures

In order to obtain a fully representative measure of possible effects of the various light régimes on stomatal behaviour, it would have been necessary to sample the plants at different times in each of the three light conditions; full daylight, reduced or artificial light, and darkness; or better still to have obtained continuous porometer records throughout the treatment sequences. Unfortunately this was not possible under the conditions, particularly in view of the large number of treatments involved. The data obtained and shown in Table XIII represent the state of opening or closing reached in each case at the end of the light treatment shown. At this point (i.e. after some hours of such conditions) complete or near equilibrium was probably attained. Even so it was not possible to gather a full set of such observations. However, there appear to be no striking differences between the different illumination treatments. In all treatments there was a substantial degree of stomatal closure by

TABLE XIII

Stomatal Apertures

	XANTHIUM				HYOSCYAMUS		
	Stomatal diameter (μ) at end of				Stomatal diameter (μ) at end of		
Treatment	Full light	Dim light	Dark	Treatment	Full light	Dim light	Dark
				Full sun	6·58	—	—
				D 24	6·58	—	—
D 8f	2·35	—	1·49	D 20	5·69	—	1·89
a	3·66						2·89
S 8a	3·80	1·14	1·43	S 20f	5·72	3·89	2·32
				a			1·14
F 8a	3·26	1·86	1·63	F 20f	5·21	2·35	2·03
				a			1·49
I 8a	3·75	1·63	1·66	I 20f	5·49	3·43	1·57
				a			1·40
D 14	—	—	1·86	D 14	5·12	—	—
S 14	—	—	1·40	S 14f	—	2·57	—
				a		2·49	
F 14	—	—	1·54	F 14f	—	1·74	—
				a		2·06	
I 14	—	—	1·40	I 14f	—	2·09	—
				a		1·63	

the end of the reduced-light treatment as well as at the end of the dark period. Taking the means of all treatments for the three light conditions (daylight, reduced light, and dark) it is seen that in the long-day plant *Hyoscyamus niger* stomatal apertures were reduced from the full-light condition to about 43 per cent. by reduced light and further to 32·5 per cent. by darkness—closure not being complete. In *Xanthium*, the short-day species sampled, stomatal apertures were reduced by low light to almost exactly the same extent, i.e. 45·8 per cent. but in this instance there was no further effect of darkness at all —at least as judged by samples taken at the end of the dark period. This difference between the two species might well be due to dark opening of stomata in plants grown under short-day conditions, such as has been shown to occur in the Chrysanthemum under similar circumstances (Schwabe, 1952).

TABLE XIVA

Xanthium. Leaf Posture

Mean Angle Subtended by the Leaf

	Leaf No.				
Treatment	5	6	7	8	Mean
D 24	160·8	106·0	92·8	78·5	109·5
D 8f	92·7	108·3	89·3	90·3	95·2
D 8a	98·5	91·7	99·7	84·8	93·7
S 8f	126·7	96·8	77·8	85·3	96·7
S 8a	95·8	97·5	86·7	78·0	89·5
F 8f	99·0	65·3	63·3	86·6	78·6
F 8a	76·0	91·0	69·0	84·1	80·0
I 8f	90·1	83·8	75·3	83·8	83·3
I 8a	112·1	98·0	90·8	88·3	97·3
Mean	105·7	93·1	82·7	84·4	91·5

Nastic responses in leaves of Xanthium

This series of measurements is also best presented separately. The curvatures of petioles and leaf blades were all observed 26 days after the start of differential treatment, at the same time of day (afternoon) in the 8-hour photoperiod and continuous-light treatments. Measurements were made on four different leaves of each plant (Nos. 5–8). Table XIV*a*, the angles subtended by the leaf (regarded as an arc of a circle), records a measure of the degree of curvature of the blade and petiole, while Table XIV*b*, the angle between petiole and stem, is a measure of epinasty at the point of insertion of the leaf.

The data have not been analysed statistically but the following effects are likely to be real; the over-all degree of leaf curvature was much greater in continuous light than in any other treatment and this was combined with a rather acute angle of insertion of the petiole on the stem, the effect being greatest in the oldest leaves. The angle subtended by the leaf does not seem to have been much affected either by intensity of illumination or by spectral composition during the low-intensity part of the photoperiod. The angle between petiole and stem, however, was largest (the average being almost 90°) when the 8-hour day consisted entirely of full daylight. This angle then diminished in the following order of treatments: S F I. Throughout all treatments the angle of insertion became consistently larger with leaf age, the mean increase amounting to about 69·5 per cent. of the value found at the time when the leaf was almost fully expanded.

Table XIVb

Xanthium. Leaf posture

Angle of Petiole with Stem (tangent at point of insertion)

Treatment	Leaf No. 5	6	7	8	Mean
D 24	65·2	85·5	60·8	55·4	66·7
D 8f	99·0	90·6	81·4	62·3	83·3
D 8a	109·0	100·9	79·3	66·8	89·0
S 8f	84·0	77·0	68·5	59·6	72·3
S 8a	87·5	72·4	65·3	59·3	71·1
F 8f	70·5	77·1	66·6	46·7	65·2
F 8a	68·8	70·2	72·4	49·4	65·2
I 8f	68·7	61·2	61·7	52·9	61·1
I 8a	67·0	55·9	55·0	48·2	56·5
Mean	80·0	76·8	67·9	55·6	70·0

Effect of reduced-light treatments on epidermal cell sizes in beet

The mean epidermal cell sizes of the young mature beet leaves sampled are recorded in Table XV together with the mean areas of the leaves from which the samples were taken. All reduced-light-intensity treatments led to large increases over the cell sizes found in full daylight, the order of cell size being D < S < F < I. Owing to the small degree of replication of the leaf samples, not all the differences reach significance level. The effects are similar for both upper and lower epidermes, though the latter had smaller cells; they parallel effects on the leaf areas themselves.

Table XV

Surface Area of Epidermal Cells in Beet

Treatment	Upper epidermis μ^2	Lower epidermis μ^2	Mean area of sampled leaf cm.^{2}
D 14f	1,443	983	15·9
a	1,240	830	17·8
S 14f	1,770	1,250	26·5
a	1,637	1,053	27·2
F 14f	2,537	1,763	28·4
a	2,483	1,310	25·8
I 14f	3,607	2,557	32·1
a	3,127	2,127	26·1

Discussion

The effects described fall into two classes, those on growth and those concerned with the flowering behaviour and photoperiodic reactions. In the case of the short-day species, *Xanthium pennsylvanicum* and *Kalanchoe blossfeldiana*, effects on flowering were relatively slight, but in the long-day plants, *Hyoscyamus niger* and the annual variety of *Beta vulgaris*, marked differences emerged between some of the light treatments. On the other hand considerable modifications of dry-matter accumulation, leaf area, &c., were found in all the four species used. It is therefore proposed to discuss these two aspects separately. This is not to imply, of course, that the effects are regarded as unrelated, but too little is known about the interrelation of growth and flowering responses to suggest immediate causal connexions.

Growth effects

In the present experiment dry weight represents the best estimate of the over-all growth made under any given condition of illumination. In the two short-day species the heaviest plants were those receiving the longest period of illumination—continuous daylight. Among the other treatments the greatest weights were found in the series receiving full daylight throughout the photoperiod. Both these effects are what would be expected (cf. also Pohjakallio and Salonen, 1947). However, in the two long-day species the corresponding differences were much less marked.

A partial explanation for the relatively high dry weights in shading and incandescent-light treatments in beet is likely to be found in the leaf-area differences induced by the low-light treatment (cf. below). Also, since continuous-light treatment in beet and henbane led to early bolting and flowering (after which leaf production ceases), the resulting restriction of dry-matter production must have contributed to all the spectral composition effects; those due to incandescent light, being most favourable in *Xanthium* and beet, can probably also be explained as mainly due to leaf-area effects. Such results were not general, however, for in *Kalanchoe* reduced daylight was optimal among these treatments.

A further effect requiring explanation is found in the dry-weight differences between treatments whose daily light period either started or ended with full daylight. Almost quite consistently those treatments whose full-daylight period coincided with the first part of the actual day yielded the heavier plants (i.e. 'a14' in *Xanthium*, 'a10' in *Kalanchoe*, 'a14' in beet, and almost significantly so 'f20' in beet). Possibly light-intensity differences may have been involved in the 14-hour photoperiod, since this was not quite symmetrically arranged around noon; but a similar explanation cannot apply to the effect in the 20-hour photoperiod, and even less to that of the 10-hour photoperiod in *Kalanchoe* which was also shifted from 12 o'clock but in the opposite direction (cf. Table I). Another possibility is that the CO_2 level in the greenhouse may have been higher in the beginning of the day than some hours later, though in view of the ventilation given at all times this does not appear to be very likely; it may be that internal factors such as stomatal opening, &c., were involved as well.

Comparing the reductions in dry weight due to decreased period of daily illumination (e.g. in the short-day species by $\frac{2}{3}$, from 24 hours to 8 hours, or by $\frac{7}{12}$ to 10 hours or due to reduction of light intensity during half the photoperiod (to at most $\frac{1}{5}$)), it is clear that the observed effects do not correspond in magnitude with these illumination differences and that much greater dry-weight differences might have been expected. In fact, the adverse effects of the reductions of light intensity and duration were compensated to a large extent by modifications in growth and metabolism, and we must look to the two factors of leaf area and the over-all efficiency rate of dry matter accumulation (N.A.R.) for the immediate explanation.

In a previous paper (Schwabe, 1953) an attempt was made to account for differences in total dry-weight production in plants subjected to various nutritional treatments in terms of N.A.R., mean leaf area, and leaf number. In Table XVI a similar analysis is presented for *Xanthium*, though it is based on rather less complete data than in the nutritional study with bracken. A similar set of figures for the annual beet is also shown in Table XVI but in this case it should be remembered that the dry weights do not include the small fibrous roots. In this table the mean values for the full-daylight treatments, disregarding the continuous-daylight sets, are shown for each sample or photoperiod and these values are then put equal to 100 per cent. All other treatments are expressed as percentages of these. As the two daylength sets were sampled on two different occasions the differences between their absolute dry weights and leaf areas reflect of course mainly their different periods of growth, but their N.A.R.s are of course comparable.

N.A.R.s are usually expressed as gm./dm.^{2} leaf/week (or mg./cm.^{2}/week as above), though other bases of reference than leaf area have been used (cf. Watson, 1952). Since the present experiment comprises treatments involving different but controlled lengths of day and periods of known lengths at reduced light intensities, it becomes both necessary and possible to make allowance for these. N.A.R.s have therefore been calculated in the usual fashion and also as mg. CO_2/dm.^{2}/hour light, calculating all dry-matter increases on the carbon content of hexose sugars. Allowance for the reduction in light intensity has also been made by assuming that such reduction to less than $\frac{1}{5}$ of daylight can be equated to a restriction of the total period of illumination of the same degree. This almost certainly undercorrects the values since the average light intensity even in the longest full-daylight treatment must have exceeded 500 foot candles and that of the low-light treatments was about 100 foot candles, though of course measurements made with an ordinary photoelectric cell are not strictly comparable for different sources of illumination. In this connexion it must be remembered that stomatal apertures were diminished to approximately 45 per cent. of the fully open condition and gas exchange must have been slowed down to some extent, though at this reduced light intensity CO_2 is not likely to become limiting. While N.A.R.s expressed on a 'per week' basis were clearly depressed in the shading- and reduced-light treatments (which is in agreement with previous work, e.g. Milthorpe (1945), Blackman and Wilson (1951*b*)), they were not so if allowance be made for light period and intensity.

TABLE. XVI. *Relative Effects of Treatments on Total Dry Weight, Leaf Areas and N.A.R.s*

	Xanthium					*Beta vulgaris*			
Treatment	Total dry weight	Total leaf area	N.A.R. mg./cm.^{2}/wk.	N.A.R. mg. CO$_2$/dm.^{2}/hr. full light [+20% for low-light treatments]	Treatment	Total dry wt. tops and tap root	Total leaf area	N.A.R. mg./cm.^{2} /wk.	N.A.R. mg. CO$_2$/dm.^{2}/hr. full light [+20% for low-light treatments]
SAMPLE (I)									
D 8 Mean values	1·417 (g.)	104·8 (cm.^{2})	3·10	8·11	D 20 Mean values	1·083 (g.)	110·0 (cm.^{2})	2·59	2·71
D 24 (1)	122·0	67·0	184·8	59·1	D 24	96·5	55·8	136·3	113·7
D 8	100%	100%	100%	100%	D 20	100%	100%	100%	100%
S 8f	87·1	113·3	67·1	112·0	S 20f	92·8	123·7	80·3	133·9
S 8a	86·5	114·6	65·5	109·4	S 20a	80·7	121·7	69·9	117·0
F 8f	85·7	113·1	64·5	107·6	F 20f	81·8	151·6	61·4	102·6
F 8a	77·5	115·2	48·1	80·1	F 20a	56·8	125·9	46·3	77·5
I 8f	89·6	120·7	69·4	115·7	I 20f	85·0	109·5	79·2	132·1
I 8a	88·3	135·3	62·3	103·9	I 20a	90·5	146·5	70·2	117·3
SAMPLE (2)									
D 14 Mean values	2·359 (g.)	157·8 (cm.^{2})	3·75	5·07	D 14 Mean values	1·094 (g.)	121·8 (cm.^{2})	2·06	3·31
D 14 as % of 1st sample	(166·5)	(150·6)	(121·0)	(62·5)	D 14 as % of 1st sample	(101·0)	(110·7)	(79·5)	(122·1)
D 24 (2)	113·6	83·3	140·0	58·8	—	—	—	—	—
D 14	100%	100%	100%	100%	D 14	100%	100%	100%	100%
S 14f	86·1	113·7	74·1	123·3	S 14f	116·5	157·4	87·9	147·1
S 14a	94·9	117·2	83·5	139·1	S 14a	139·6	148·3	110·7	185·2
F 14f	83·3	125·0	65·3	108·9	F 14f	88·3	150·1	67·5	112·7
F 14a	99·7	122·1	87·2	145·0	F 14a	127·9	152·4	99·0	165·6
I 14f	83·2	120·5	66·7	110·0	I 14f	122·1	206·6	77·2	129·0
I 14a	100·9	123·3	88·5	147·3	I 14a	144·9	157·5	110·7	185·2

On their face value the N.A.R.s adjusted in this manner suggest that plants receiving shorter daylengths are more efficient than those in the longer photoperiods, i.e. in *Xanthium* plants in full daylight and 8-hour photoperiod the increase amounts to 60 per cent. over a 14-hour photoperiod; in beet the increase in a 14-hour day over a 20-hour day is 22 per cent. Unfortunately, however, with diurnal changes of light intensity—even if largely damped in the Arctic summer—it is not possible strictly to compare different photoperiods directly, and it seems probable that a considerable part of the apparently greater efficiencies is due to a higher average light intensity in the shorter photoperiods of these particular treatments. This may well explain also the somewhat lower value found for beet in the 20-hour day compared with continuous light since in the former treatment the 4-hour dark period coincided with a time of high light intensity (10 a.m. to 2 p.m.). None the less, even if no allowance at all be made for differences in the lengths of photoperiod, the N.A.R.s of the *Xanthium* treatments in a 14-hour day, for instance, are only 21 per cent. greater than those in the 8-hour day, and it might have been anticipated that the extra 6 hours of light would have contributed to more than this amount.

Differences in assimilation rates in different daylength treatments have been noted by Bode (1942), increased rates being found in *Kalanchoe* when given short-day conditions. Dark fixation of CO_2 in short-day treated plants may have shifted the balance in the same direction (cf. Gregory *et al.* 1954). Or again the effect might represent a diurnal decline of assimilatory efficiency from a high level at the beginning of the light period, possibly even as a consequence of a period of active assimilation. Diurnal variations in dry-weight accumulations have been noted in a number of papers, some of which are quoted by Goodall (1945), who also noted highest dry-weight accumulation in the morning. Such changes might also serve partly to explain the differences between plants receiving the high- and low-light portions of their photoperiods in different orders, though these differences are not sufficiently consistent between the two samples to give much support to this suggestion.

It is easier to make comparisons between the different light treatments within each photoperiod, and the data adjusted to mg. CO_2/dm.^{2}/hour full light plus 20 per cent. would seem to indicate that here also greater efficiency contributed towards compensating for the reduced number of hours at full light intensity. This applies especially strongly to the S and I treatments.

The difficulties of validly interpreting N.A.R. values from an experiment of this kind serve as a reminder of how much more satisfactory direct measurements of assimilation and respiration rates would have been.

Leaf area is the second factor responsible for the far-reaching compensation of the effects of reduced light intensity and duration of the illumination. Comparison between the leaf areas of plants grown in continuous light and those of plants in other photoperiods demonstrates the striking effect of even a few hours of darkness every day on leaf size. In *Xanthium* total leaf areas were increased by 20 per cent. in a 14-hour photoperiod and by 49 per cent.

in the 8-hour photoperiod relative to those attained in continuous daylight treatments. In beet as little as 4 hours of darkness per day increased leaf areas by 80 per cent. over those produced in continuous light; in *Hyoscyamus* the effect reached 40 per cent. and even in the day-neutral tomato the leaf areas in a 16-hour day were 32 per cent. greater than in a 24-hour day.

The magnitude of the increases found in those treatments receiving half their photoperiods as low-intensity daylight is also strikingly large, increases ranging from about 15 per cent. in *Xanthium* to 23 and over 50 per cent. in beet in 20- and 14-hour photoperiods. Fluorescent light caused yet further increases especially in beet and *Hyoscyamus*, while in beet maximum leaf areas were found with incandescent light; in *Xanthium* the increase amounted to about 25 per cent. above full daylight in both photoperiods, in beet it exceeded 80 per cent. in a 14-hour day. These large increases in leaf area due to variation in length of photoperiod and/or light intensity during part of the photoperiod are particularly interesting for another reason. It is well known from many previous researches, including the most recent series of papers by Blackman and co-workers (e.g. 1954), that reduction of light intensity causes increases in leaf area. The term 'shade leaves' has been used to describe fully developed examples of this kind, especially if they exhibit anatomical differences also. It is clear from the present results that such enlarged leaves can also develop when a substantial part of the daily photoperiod consists of full day- or sun-light, e.g. 7–10 hours in beet.

From the leaf numbers known to be present at the beginning of differential treatment it is possible in *Xanthium* to discover at what age of leaf it becomes impossible to modify its area by changes in the conditions of illumination. In this case, at the time of the change in treatment, leaf No. 7 had just separated from the apical bud and leaf No. 6 was expanding its blade; cell division in the latter had most probably ceased altogether. Yet when finally expanded leaf No. 6 was already significantly larger in the shorter daylength, and beginning with leaf No. 7, in which cell division must also have been almost, if not completely, ended, nearly all effects on leaf area reach statistical significance. This result would seem to indicate that the environmental effects on leaf size are not due primarily to altered numbers of cells but rather to changes in cell size, a suggestion which is supported by the cell measurements actually carried out with beet. These measurements of the areas of epidermal external cell walls were made on both the upper and the lower leaf surfaces in all treatments having a 14-hour photoperiod. Knowing the areas of the individual leaves from which the samples were taken, a regression was calculated of leaf size on epidermal cell size and the regression was found to be highly significant (Fig. 4, *a* and *b*). Treatments leading to the production of large leaves also produce large epidermal cells. Moreover the areas of the sampled leaves are proportionally in good agreement with the corresponding epidermal cell areas. It seems safe therefore to conclude from these two different methods of approach that the differences in leaf size are primarily due to increases in cell sizes, and not in cell number. It may be noted in this connexion that the cell sizes were all measured on young fully expanded leaves from the various treatments and do not represent a random sample of leaves of all ages.

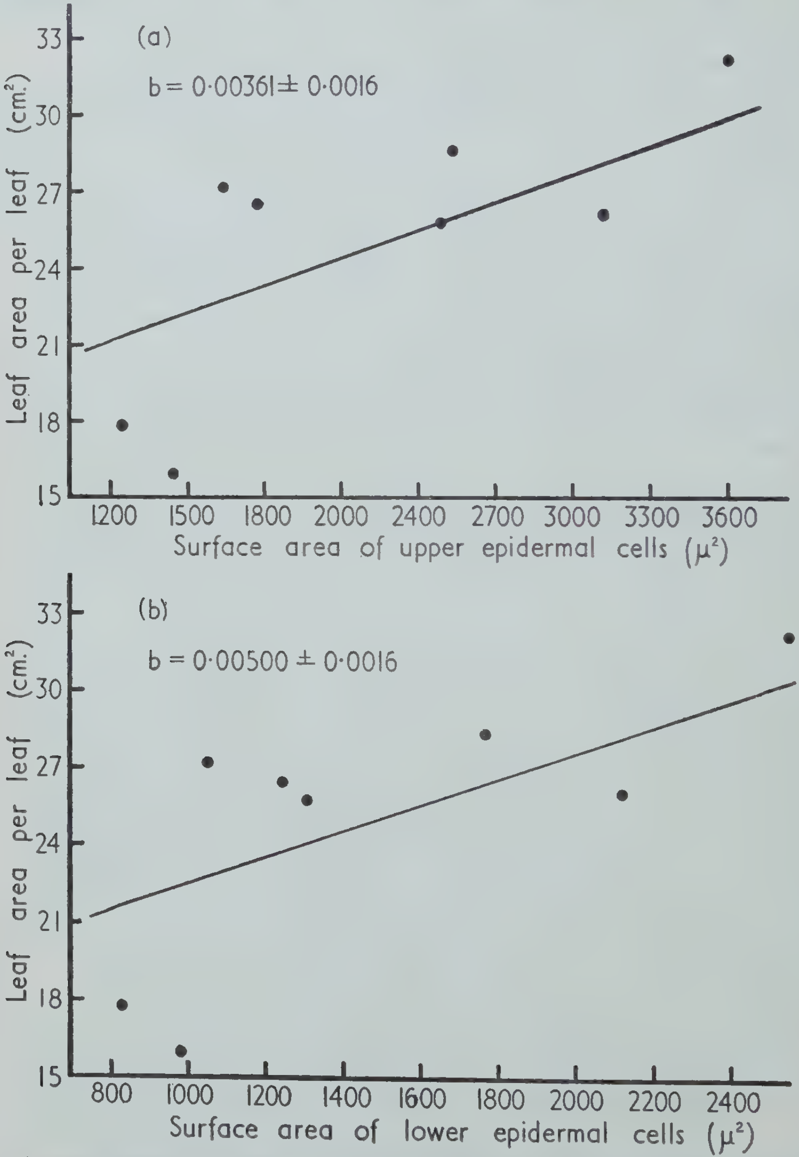

FIG. 4. Relation between leaf area of beet and cell sizes (area of surface of external cell walls); (*a*) upper and (*b*) lower epidermis. Regression line of leaf area on mean cell surface area is also shown.

The close correlation between epidermal cell size and leaf area is presumably bound up with water content which also shows a positive correlation with total leaf area. Regressions of leaf area on water content give significant results in *Xanthium* (both samples), *Hyoscyamus*, and beet (20-hour day); these are shown in Figs. 5–6.

Morton and Watson (1948) also determined cell sizes and numbers in beet grown under different nutritional conditions; and in a preliminary experiment in light and darkness defoliated and normal plants were compared. Their finding that cell size is much affected by defoliation, which they attribute to differences in water-supply, agrees very well with the present data.

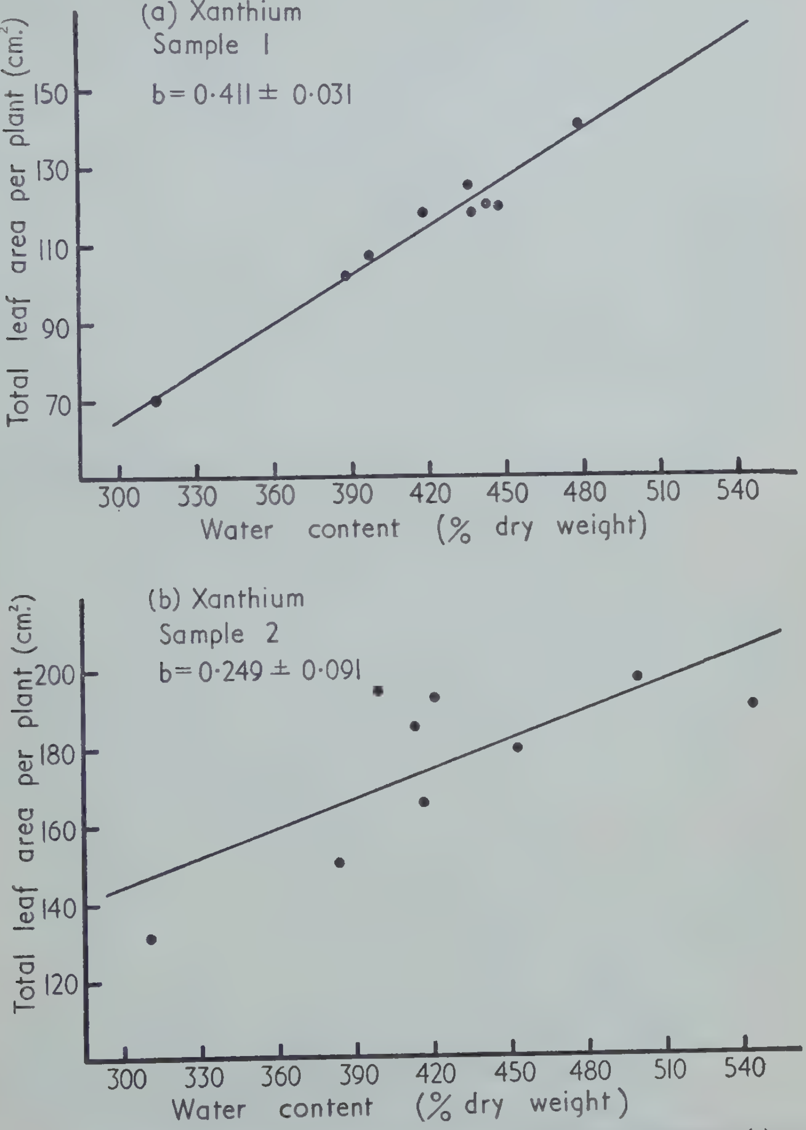

FIG. 5. Relation between water content and leaf area in *Xanthium pennsylvanicum*, (*a*) sample 1 and (*b*) sample 2. Regression lines of leaf area on leaf water content are shown.

It would be interesting to know the mode of action of darkness or reduced light intensity in increasing cell size. Two possible causes come to mind. It may be that under conditions of continuous light transpiration rates remain relatively high throughout the 24 hours, the stomata remaining open all the time; the plants may thus never attain full turgor in spite of the soil being kept

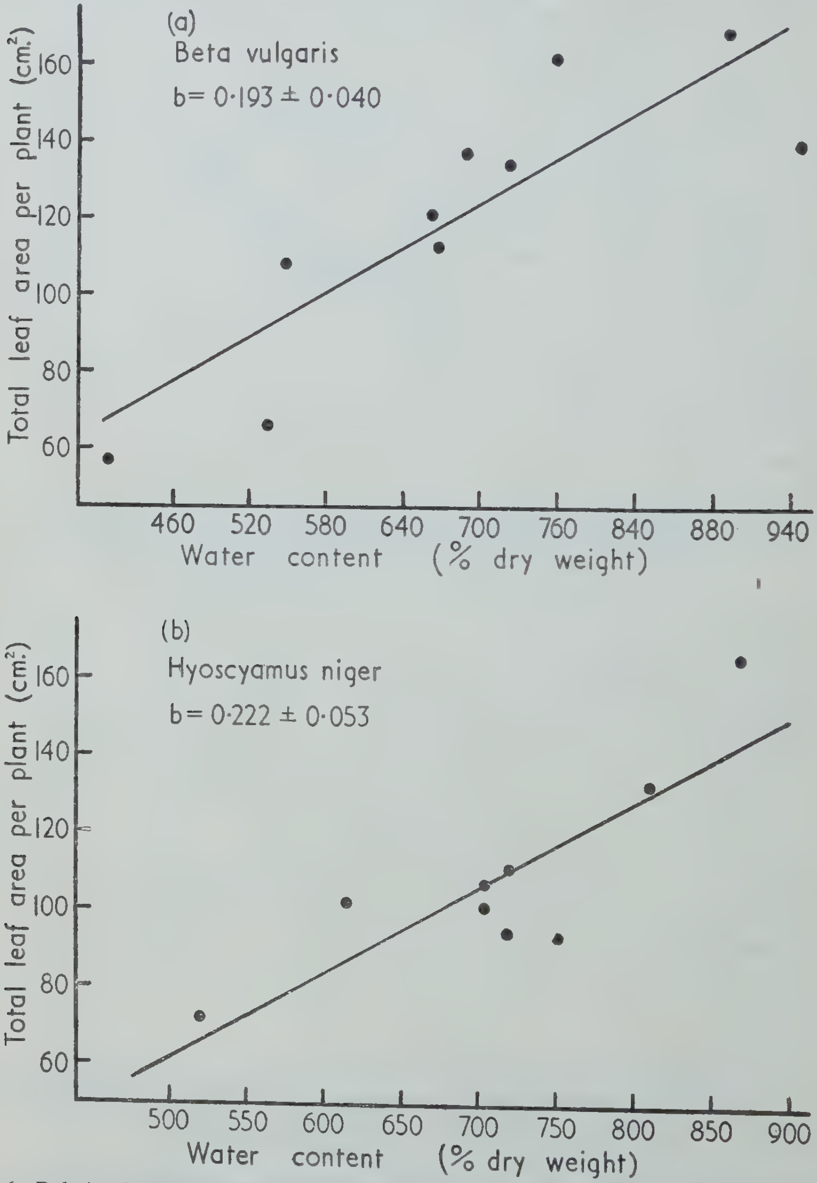

FIG. 6. Relation between water content and leaf area in *Beta vulgaris* and *Hyoscyamus niger*, (*a*) beet sample 1 and (*b*) henbane sample 1. Regression lines of leaf area on leaf water content are shown.

well watered at all times. With some period of darkness or in reduced light, transpiration may fall temporarily to a low level and the fully turgid condition may then be reached.

Thut and Loomis (1944) determined stem and leaf growth over periods of 2–3 days in several species under natural conditions and concluded from their results that 'growth' measured by an auxanometer was not inhibited by light as such, but was the resultant of positive temperature effects and negative effects on water balance. Although these short-period observations may not be directly relevant to a study of final leaf sizes, the emphasis placed on water balance is in harmony with the above conclusions. However, in view of the controversial position concerning the relation between turgor and cell expansion the question must be left open.

The second possible cause is to be sought in differential effects of the kinds of illumination on growth-hormone metabolism. This is suggested also by the effects of different light sources on height increments in *Kalanchoe* and *Xanthium*. The tallest plants, i.e. those receiving incandescent light, also had generally large leaves, though leaf-area differences were not proportional to differences in heights.

The differential effects of the various light treatments on leaf curvature and epinasty in *Xanthium* also support this suggestion. While one can only guess at the auxin content of the leaves, the curling found in continuous light appeared rather similar to that in plants sprayed with hormone solutions. If for the sake of argument the height attained by a plant be taken as a measure of its hormone content, it would appear that as far as light intensity is concerned again a correlation exists between hormone content and leaf size; but this correlation does not account for any effects of dark periods of increasing lengths, short-day plants being shorter with larger leaves, not taller. Of course subsidiary hypotheses might be made to accommodate these results also, but these would be valueless in the absence of any direct evidence. The data of Stolwijk (1952) and Wassink and Stolwijk (1952) also suggest that at least as far as long-day plants are concerned the 'degree of flowering' is positively correlated with those conditions which lead to maximum internode elongation. In their highly interesting investigations the authors have studied the effects of bands from different parts of the spectrum, on growth and flowering in several species, when used either as the only source of illumination or as a supplement to a period of illumination with white light. Growth was generally measured on the basis of internode elongation (stem length), but dry weights were also determined in some instances. The authors found remarkable differences between the effect of light of various wavelengths. In tomatoes, for instance, if 8 hours under white light were followed by 8 hours in coloured light, the longest plants were produced by blue and infra-red light. In monochromatic light, blue produced the shortest and red the tallest plants, green and yellow being intermediate in this respect. When *Cosmos* was grown in the same wavebands, the red-light plants were more than four times as heavy as the blue-light series. Clearly, however, in all these results the purely morphogenetic effects must be confounded with the effects on photosynthesis and therefore on the substrate available for growth, much in the same way that auxin action on coleoptile elongation can be enhanced by supplying soluble carbohydrate. It must be admitted with regret, therefore, that unfortunately the technical difficulties are too great to separate these effects satisfactorily. The authors state that although greater, the number of quanta received in the red does not suffice to explain the large dry-weight differences. It is thought that auxin effects must ultimately be involved also, but no single mechanism can at present be suggested. It would be interesting in this connexion to know how leaf areas, assimilation, and respiration rates &c. are affected by these different spectral regions. Such a study might go some way at least towards distinguishing between morphogenetic and other effects.

It seems likely that the large effects noted in incandescent light in the present experiments were due in no small measure to the infra-red content of the radiation from this source, though the high proportion of red must also have contributed to the effects.

To sum up this discussion it is found that the high degree of compensation for reduction of light intensity and photoperiod was brought about mainly by increased leaf area and probably also by a higher N.A.R. in the plants so affected. Growth and development of the plants subjected to these conditions were therefore modified so as to minimize the effects of the less favourable environmental conditions, as was hinted in Gregory's pioneering analysis of leaf growth (1926, p. 20). It is particularly interesting to note from the present experiments that the same responses to reduced-light conditions &c. were evoked when these conditions were given for only a part of the daily photoperiod as when they were continuous. The increased leaf areas themselves were due to increased cell size and related to the water content of the plants. These differences it is suggested may represent the results of internal changes of turgor and/or hormone levels.

Flowering effects

In the two short-day species alteration of light intensity and spectral composition during half the photoperiod had relatively little effect. This, however, should not be taken as evidence for lack of sensitivity in these species; the reason is more likely to be that both the daylengths used were rather too favourable for any differences to show up. The effect of low-intensity illumination noted in *Kalanchoe* (Schwabe, 1954) in other experiments is also seen in the present data. In the plants receiving reduced or artificial light in an 8-hour day the mean number of flowering axes was reduced by 24 per cent. compared with the full-light controls; in a 10-hour day the reduction was 18 per cent. In the previous experiment quoted the reduction under somewhat similar conditions was about 50 per cent. No effect on the time to macroscopic budding was evident in *Xanthium* in the present experiments.

In the long-day plant annual beet illumination with fluorescent lamps failed almost completely to evoke the long-day response, and this is much the most striking effect on flowering found in the present experiments. Even when given 10 hours of full daylight followed by fluorescent light for a further 10 hours the result was the same as under short-day treatment. It is of interest also that in the annual beet in this treatment series relatively low dry weights and net assimilation rates were found, but only when the fluorescent-light treatment followed the full-daylight period, not when it was given first. In a 14-hour day lower weights &c. resulted when the fluorescent light came first in the photoperiod; this inconsistency renders it unlikely that the fluorescent light was insufficient for assimilation and of too low an intensity to be perceived at all by beet. Since these experiments were carried out, Borthwick and Parker (1952) have noted the same effect with this particular plant. From their researches into the photoperiodic action spectra of a number of species (Borthwick, Parker, and Hendricks, 1948) it is clear that the sensitivity of many plants to photoperiodic stimulation reaches a peak in the red part of the spectrum. It would appear therefore that the relative shortage of radiation in this part of the spectrum from fluorescent lamps (even in the 'daylight matching' type used) was the reason for the failure of beet to flower under these conditions. If this is correct it seems possible that this long-day species might even be maintained in continuous light without evoking the flowering response to long day. The behaviour of barley resembled that of beet though flowering was not completely suppressed in this instance.

Iberis—remaining quite unaffected by the deficiency of red radiation—behaved much as would be expected of a *crucifer* according to Funke's classification (1939).

Hyoscyamus niger, the other long-day plant used, also responded to the light quality in its flowering behaviour, but fluorescent light merely caused a delay in its bolting compared with the other light treatments. Again, lack of red radiation would seem to be the cause, especially in view of the significant acceleration of flowering when red-rich incandescent light was used for the half photoperiod.

It would appear probable that these effects of wavelength involve the growth hormone metabolism in some manner (cf. also Liverman, 1955). The effect of low light intensity hastening bolting in *Hyoscyamus* and beet, also strengthens the idea that under these conditions higher growth-hormone levels are attained (cf. Schwabe, 1954) which in the case of *Hyoscyamus*, at least, may favour flowering (cf. Liverman and Lang, 1952, who induced flowering in this species by auxin application).

The importance of the order of the high- and low-light treatments in the *Hyoscyamus* series in a 14-hour day also requires explanation. The significantly earlier bolting with low light intensity at the beginning of the photoperiod agrees well with the observations made by v. Denffer (1939), Claes (1947), and Fabian (1938–9). In the henbane there appears to be no correlation with growth effects and no explanation of the phenomenon is offered.

CONCLUSIONS

These experiments indicate that both the light source used to supplement natural daylight and the intensity of illumination have pronounced effects on the structure and growth of the plants so treated, effects which cannot be neglected in photoperiodic experiments, especially perhaps where artificial light sources are used as the sole means of illumination. The modifications of growth tend in general to be in a direction which minimizes the adverse effects of the reduced light intensity or duration. This compensation is achieved mainly by increase of the leaf-cell size and thus of the leaf areas, but probably also by a greater over-all 'efficiency' in dry-matter production if the total period and intensity of illumination be taken into account. The long-day response of sugar-beet, barley, and *Hyoscyamus* may be almost completely suppressed if the relatively low-intensity 'white' light used as a supplement is deficient in the red end of the spectrum.

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Polarized Segregation in *Bombardia lunata*

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With one Figure in the Text

ABSTRACT

Data for the arrangement of ascospores in *Bombardia* collected by Zickler (1934) and analysed by Catcheside (1944) suggested the polarization of segregation of a single gene affecting spore colour. The conclusion has been re-examined from crosses between wild type and the *rubiginosa* mutant under conditions where the environment was controlled and precautions were taken to eliminate bias introduced by the observer. The same polarization was found to exist within the first-division segregation classes but to a lesser degree while the bias between the symmetric and asymmetric second-division classes is exactly reversed. Cytological observations indicate that the plane of the second division is not preserved in the ascus. It is concluded that the apparent polarization probably occurs after meiosis where segregation is normal.

INTRODUCTION

BOMBARDIA LUNATA Zckl. is a coprophilous ascomycete belonging to the family Sordariaceae in the order Sphaeriales. It was isolated by Zickler from horse dung, and described by him as a new species (Zickler, 1934). He investigated its morphology and life-history in considerable detail and also carried out genetical studies using certain spontaneous and radiation-induced mutants. The fungus shows both physiological and morphological heterothallism. The former system is controlled by a pair of alleles and the latter is the result of mutations affecting the production of sex organs which give rise to male strains in contrast to the wild type which is hermaphrodite. Zickler also described strains which were exclusively female, but these have not been encountered in the present work. There are no asexual spores, but colourless uninucleate spermatia are produced in flask-shaped spermogonia which are developed laterally on the hyphae. These spermatia are incapable of germination to form a colony and they function solely as fertilizing agents.

Zickler dealt with a number of mutants affecting the growth form of the fungus and a further series affecting both the colour of the mycelium and the ascospores. This enabled him to score segregation without dissecting asci and consequently to amass a large volume of data with relative ease. From these data he postulated that homologous centromeres divide at the first division of meiosis in the ascus, with segregation of the four chromatids at random. As a consequence, all loci would be expected to show second-division segregation in 66·7 per cent. of cases and this Zickler claimed to be so in *Bombardia*.

In spite of this claim data were given on the segregation of both colour and

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mating type in which the percentage of second-division segregation was significantly less than 66·7. Zickler found that colour and mating type were linked but was unable to reconcile the percentage recombination with the percentage of second-division segregation of the two loci and again argued that recombination with the centromere cannot be explained by crossing over.

Ryan (1943) has shown that Zickler failed to consider and allow for the occurrence of double cross-overs, and if this is done the data agree with expectation.

Zickler did not consider the frequencies of the six different arrangements of spores in asci where spore colour was segregating. Catcheside (1944) pointed out that in Zickler's data these frequencies differ significantly from expectation on the basis of random segregation. In a cross between wild type and the mutant *rubiginosa* there appeared to be a tendency for the wild type spores to be at the top of the ascus in a majority of cases when the factors for colour segregated at the first division. A similar bias appeared to be present in the second-division segregation classes, the wild type allele again showing a tendency to segregate to the upper position. A similar phenomenon was found in data from a cross between wild type and the mutant *lactea*. As Catcheside pointed out, this may be due to an unconscious selection on the part of the observer or it may be caused by some material on the chromosome bearing the mutant locus which is differentially oriented by a gradient in the ascus.

In 1948, cultures of *Bombardia* were sent by Professor Laibach at Frankfurt to Professor Catcheside and an attempt was made to determine whether the polarization was real, and if so what caused it.

MATERIAL AND METHODS

(*a*) *Stocks.* Of the two cultures received from Germany, one was apparently a cross between two cultures having the colour of the wild type and contained a few ripe perithecia from which the two mating types were isolated; the other was a culture of the *rubiginosa* mutant which was labelled *A*. Presumably this referred to the mating type and this culture was used as a standard of reference.

Zickler (1934) used 2 per cent. 'Biomalt' agar as a medium for all his work. It appeared desirable to reproduce conditions as accurately as possible if comparisons were to be made with his data, therefore all cultures were grown on medium consisting of 2 gm. malt extract, 1·5 gm. agar in 100 ml. distilled water, autoclaved for 5 minutes at 15 lb. pressure.

Conditions for growth and particularly for sexual reproduction of *Bombardia* have been found to be comparatively critical. It will not grow on a minimal medium consisting of mineral salts, inorganic nitrogen, with glucose or sucrose as a carbon source. Supplements to this medium of a solution of the B vitamins, vitamin-free hydrolysed casein, tryptophane, cystine, tyrosine, nucleic acid, hydrolysed nucleic acid, adenine, adenilic acid, uracil, and hypoxanthine similarly failed to support growth. It grew vegetatively on minimal medium supplemented with yeast extract and grew adaptively on minimal supplemented with hydrolysed casein which was known to contain peptide factors. For both vegetative growth and for sexual reproduction malt extract agar proved to be superior to any other medium tested.

The optimum temperature for vegetative growth was 22° C. and for development of perithecia 18–20° C. At 22° C. relatively few perithecia were formed and at 25° C. perithecial development was completely suppressed. The generation time at 20° C. is 5–6 weeks.

When asci are mature the spores may either be forcibly ejected or as usually happened may be set free inside the perithecium. At 22° C. the ascospores frequently started to germinate inside the ascus even before the walls had broken down.

(*b*) *Crosses*. Crosses were made in 9 cm. petri-dishes containing about 20 ml. malt agar. Ripe perithecia were not formed until 8 weeks after inoculation of the two strains but this time could be shortened to 6 weeks by placing several inocula on the same plate.

Alternatively crosses may be made by spermatizing protoperithecia of one strain with spermatia from the other. Malt-agar plates were inoculated at four equidistant points with one strain and incubated for 4 weeks at 22° C., by which time protoperithecia were well developed throughout the mycelium. Spermatia were taken from a tube culture of the other parent, suspended in 2 per cent. malt extract and drops of this suspension spread over the surface of the plate culture. Such plates were then incubated at 20° C. and perithecia ripened within 14 days. All mating type tests were done in this way and between 50 and 60 cultures could be tested on a single plate. After 7 days it was possible to determine a positive or negative reaction.

Growth-rate was measured as the average increase in colony diameter in 24 hours on 2 per cent. malt agar at 22° C.

(*c*) *Dissection of asci*. Asci were dissected with a de Fonbrune micromanipulator but it is possible to dissect asci freehand under a binocular at 50–100× magnification provided precautions are taken to ensure sterility.

DESCRIPTION OF WILD TYPE AND MUTANT STRAINS

(*a*) *Wild type*. Single ascospore isolations were made from the original wild type culture. Most of these were hermaphrodite, producing abundant protoperithecia and spermatia, and appeared to be similar to Zickler's strain *bulbosa*. The mycelium and the ascospores were dark olive-green and the growth-rate was 3·8 mm. per 24 hours. This will be taken as the wild type. One of the single ascospore isolates was female sterile. It was paler in colour than the wild type and characteristically regions of the colony were black and moist in appearance. After two to three serial transfers the wild type reverted to this female sterile form and it was found that continual reisolations must be made from ascospores to maintain the hermaphrodite condition.

(*b*) *Rubiginosa*. (*r*) This is a mutant described by Zickler which affects the colour of the mycelium of the perithecia and of the ascospores, all of which are reddish-buff. The culture from Germany was female sterile, and the growth-rate was 3·2 mm. per 24 hours. Crosses between wild type and *rubiginosa* gave rise to asci in which spore colour segregated in each ascus in a ratio of 1:1. Asci were dissected and the hermaphrodite *rubiginosa* was extracted. This was found to be as unstable with respect to female fertility as the wild type. Ripe perithecia were not readily obtained from crosses when both parents were *rubiginosa*, and then only after 8–12 weeks.

Segregation of spore colour could be observed directly in the ascus and for 13,487 asci there was 63·83 per cent. second division segregation, which is close to Zickler's figure of 64·0 per cent. for 8,204 asci.

(*c*) *Albino* (*al*). This mutant arose spontaneously as a sector in a female sterile culture of *rubiginosa*. The mycelium, the perithecia, and the ascospores are colourless and the colony on malt agar is slightly moist and milky white. Phenotypically it is similar to Zickler's *lactea*, which on the basis of its origin as a sector in a *rubiginosa* culture and on the percentage second-division segregation, he regarded as allelic with *rubiginosa* although there is no record of a cross between them.

This albino culture was crossed with wild type and three different types of asci were produced: (1) 4 wild type : 4 *albino*, (2) 4 *rubiginosa* : 4 *albino*, and (3) 2 wild type : 2 *rubiginosa* : 4 *albino*. Presumably the albino mutant differs from wild type in two different genes one of which is the *rubiginosa* gene and the other the *albino*, the latter being epistatic to the former. On this basis the predicted genotypes of these three classes of asci would be:

(1)	(2)	(3)
$al^+ \ r^+$	$al^+ \ r$	$al^+ \ r^+$
$al \ r$	$al \ r^+$	$al^+ \ r$
$al^+ \ r^+$	$al^+ \ r$	$al \ r^+$
$al \ r$	$al \ r^+$	$al \ r$

These predictions were confirmed by appropriate crosses to wild type. Since *al* is epistatic to *r*, two of the seven classes of asci expected in a two-gene segregation are indistinguishable, viz. class 3 where only one gene segregates at the second division and class 7 where both genes segregate at the second division. Since in both classes half the spores are recombinant the percentage recombination can be estimated. *Al* showed 45·2 per cent. recombination with *r* in 231 asci. This is significantly different from 50 per cent. ($\chi^2_1 = 4\cdot09$), which suggests they may be on the same chromosome but on opposite sides of the centromere. *Al* segregated at the second division in 45·38 per cent. of 3,878 asci, so *al* is closer to the centromere than is *r*. This suggests that *albino* is different from Zickler's *lactea*.

(*d*) *Mating type and rubiginosa.* Mating type segregated at the second division in 63·4 per cent. of cases in 184 asci. This value is in close agreement with the average of all Zickler's data which is 63·13 per cent. Zickler stated that the loci for mating type and *rubiginosa* were linked. Data (Zickler, 1937) from 5 separate crosses (Table I), the parents of which are not given, show a range of values for recombination from 38·7 to 52·5 per cent. The first three are significantly different from 50 per cent., the fourth is significant at 0·05 but not at 0·01 whereas the fifth does not differ from 50 per cent. Data from his 1934 paper are insufficient to apply a test of significance, but values of 35·9 and 38·3 per cent. are given.

TABLE I

Zickler's (1937) data for Segregation of Colour and Mating Type

Set	No. of asci	2nd Div. Segregation % Colour	2nd Div. Segregation % Mating Type	Recombination %
1	40	50·0	59·4	38·7
2	53	55·1	61·6	40·3
3	86	55·9	65·2	39·3
4	97	60·8	62·2	45·1
5	39	71·7	69·7	52·5

Data from the crosses *rubiginosa* × wild type and *albino* × wild type are given in Table II. There is no evidence of linkage between mating type and either of the loci affecting colour. The discrepancy between the data in Table II for *rubiginosa* and mating type and those of Zickler for presumably the same two loci is difficult to explain, unless Zickler used different colour mutants without determining allelism.

TABLE II

Crosses of Different Parents with Wild Type

Parents	No. of asci	% 2nd division segregation Mating type	% 2nd division segregation Colour	P.	N.	T.*
+ × *rubiginosa*	67	57	71	10	10	47
+ × *albino*	36	50	41	8	10	18

* P.= parental ditype. N.= non-parental ditype. T.= tetratype.

NUCLEAR DIVISIONS IN THE ASCUS

Squash preparations were made and stained with aceto-carmine or acetic orcein, the latter according to the method of Kurnick and Ris (1948).

At Metaphase I the seven bivalents lie on a spindle which is parallel to the long axis of the ascus. At metaphase II the daughter nuclei are 25–35μ apart and the spindle axes are only slightly inclined to a transverse plane. The sequence of events after telophase II is important when considering polarized segregation. The two pairs of nuclei at each end of the ascus come to lie side by side each with an umbrella-shaped appendage directed towards the centre of the ascus. Before the third division the nuclei must move apart since the third-division spindles may be either close together in pairs at each end or more uniformly distributed along the length of the ascus. They may be oriented in any direction and frequently overlap. For this reason it is not safe to isolate the spores in sister pairs from the ascus; each spore must be germinated separately. Walls are finally formed round the eight nuclei present after the third division. Each spore is half-moon shaped with the convex face towards the centre of the ascus: a single tail-like appendage is cut off at the basal end of each spore and after the division of the spore nucleus one of the daughter nuclei passes into this tail.

It is obvious from the above description that the order of the tetrad nuclei is not determined by the second meiotic division although the plane of the first division is fixed. Presumably it is a matter of chance which of the two daughter nuclei at each end of the ascus moves toward the middle at metaphase III. On a purely random basis an actual symmetric second-division segregation may give rise to an apparent symmetric or asymmetric type.

POLARIZED SEGREGATION

1. *Method of Scoring*

There are a number of sources of error in scoring the frequencies of the different classes of asci which may give rise to spurious bias in the segregation. These errors may be introduced directly by the observer as a result of the methods of observation and scoring used, or they may be the result of the nature of the material.

(*a*) *Mounting.* Since the period during which the contents of a perithecium are in a suitable condition for mounting and counting the ascospore arrangements is relatively short, some method of making preparations of asci permanent or semi-permanent is desirable. Many of the usual mounting media such as dilute glycerine or lacto-phenol had the effect of causing the ascus wall to disintegrate and the spores to be set free. Mounting in water instead of 2 per cent. malt extract had a similar effect by causing the asci to burst. Gum chloral (Bolles Lee, 1937) in which material may be mounted from water was found to be suitable. To prevent shrinkage of asci the solution was diluted with twice its volume of 2 per cent. malt extract in water.

The contents of different perithecia were mounted on separate slides and examined under 1/6 objective and 10× eyepiece, using a mechanical stage.

(*b*) *Errors in observation.* The principle was adopted of making a genuine attempt to score every ascus from every perithecium examined, since it is conceivable that certain arrangements may attract the eye of the observer more than others, e.g. those with four dark spores at the top of the ascus, with the result that these would appear to be in excess.

During the separation of the mass of asci from a perithecium into smaller clumps for counting, some of the asci are dislodged from the ascogenous tissue and lie singly in the mounting medium. Such asci were only scored if the base of the ascus could be distinguished. In *Bombardia* there is a relatively short stalk which often shrinks when the ascus is free. The two first-division segregation classes and asymmetric second-division segregation classes can only be scored when the stalk is visible, but the two symmetric classes can be scored irrespective of whether the stalk can be distinguished or not. Unless the above rule is adopted a bias could be introduced which favoured classes 3 and 4.

Only asci containing eight spores were scored. Theoretically if six were present and the base and apex of the ascus could still be distinguished, it would be possible to score the ascus since the missing spores would have come from either the base or the apex. It is possible, however, that the spores may have escaped from the apex and the remaining spores moved up, thus making it appear as if the missing spore pair was originally at the base.

(*c*) *Errors due to the nature of the material.* If certain arrangements of spores in the ascus mature more quickly than others so that they can be scored while others are still immature, such arrangements would be in excess. Since the spores at the apex of the ascus mature first, an excess in classes 1, 3, and 6 might be expected. An error would only be introduced, however, if all counts were made on material which was at the same stage of development, but if perithecia were taken at different stages of development it should be possible to detect effects due to differential maturation.

(*d*) *Estimation of maturity.* The method adopted for measuring the degree of maturity of a perithecium is not ideal but seemed to be the only quantitative method available.

It was assumed that the proportion of the total asci in a perithecium which could be scored would increase as the perithecium aged; this proportion was expressed as a percentage and called the index of maturity. It is improbable that the proportion of the total asci which reach maturity at any given time will bear a direct relationship to the actual age of the perithecium for the following reasons:

(i) The proportion of immature asci would need to remain constant, the number reaching maturity being balanced by the number developing from the ascogenous hyphae, and this is probably not the case. It is more likely that the number of immature asci gradually decreases with increasing age.

(ii) The asci which have reached maturity continue to develop and finally the ascus wall breaks down so that such asci can no longer be counted. The apparent number of mature asci will therefore be less than the number that have matured in the perithecium and the discrepancy between the apparent and actual values will probably increase with age. The relationship these opposing tendencies bear to each other is not known, and therefore the index of maturity of each perithecium can only be considered to give some approximate idea of the age.

It was found that the figure obtained ranged from 14 to 65 per cent. This may be due either to the asci which are becoming over-ripe equalling or exceeding the numbers reaching maturity at this stage, or to perithecia older than this containing so many overripe asci that separation of the remainder is impossible. Mature ascospores tend to germinate inside the perithecium, the contents of which become an entangled lump.

2. *Rubiginosa* × *Wild Type*

(*a*) *Data.* Two sets of data from crosses between *rubiginosa* and wild type are given in Table III together with Zickler's totals for comparison.

The contents of each perithecium were recorded separately and for Set A classes 1 and 2 were treated as a $2 \times n$ contingency table and heterogeneity χ^2 calculated according to the method of Brandt and Snedecor. χ^2 was partitioned in five ways. There was no significant heterogeneity at the 5 per cent. level: (1) within the whole experiment, (2) between perithecia in each cross, (3) between the 10 crosses, (4) between hermaphrodite v. male, and (5) between reciprocal crosses. Classes 1 and 2 differ significantly from equality but the difference is reduced from 14·06 per cent. of the total in Zickler's data to 6·76 per cent. in mine. Furthermore the difference between the symmetric and asymmetric second-division segregation classes is exactly reversed in my data from that in Zickler's. Set B was tested for heterogeneity in the whole experiment and it was not significant. This set agrees with Set A.

TABLE III

Data for Frequency of Ascospore Arrangement in Crosses between Wild Type and rubiginosa *in* Bombardia lunata

Ascus class	Spore arrangement*				Zickler's data		Author's data			
							Set A	Set B	Total	
1	r	r	+	+	1687	†	1604	995	2599	‡
2	+	+	r	r	1271		1403	876	2279	
3	+	r	r	+	1174	2482	1357	931	2288	4515
4	r	+	+	r	1308		1394	833	2227	
5	+	r	+	r	1246	2764	1182	809	1991	4094
6	r	+	r	+	1518		1288	815	2103	

* r = *rubiginosa*; + = wild type. From left to right = from base to apex of ascus.
Classes 3 and 4 = symmetric second-division segregation.
Classes 5 and 6 = asymmetric second-division segregation.
† $\chi_1^2 = 58{\cdot}2234$.
‡ $\chi_1^2 = 20{\cdot}9922$.

(*b*) *Effect of maturity of the perithecium.* Sets A and B were grouped together and then divided arbitrarily into frequency classes according to the maturity of the perithecium. The ratio Class 2/Classes 1 and 2 is plotted, together with confidence limits, against maturity (Fig. 1).

(*c*) *Chromosomal differences.* It has been shown for two cases of polarized segregation reported in the literature that the effect is related to heterochromatin on the chromosomes concerned (Rhoades, 1942; Longley, 1946; Sturtevant, 1936). The chromosomes in *Bombardia* are too small to detect such differences cytologically unless they are very marked, but certain abnormal asci were found in crosses between wild type and the colour mutants where 3+ : 1 mutant and also 3 mutant : 1+ spore pairs occurred, instead of the regular 2 : 2 segregation. Whether these are the result of mutation at the locus for colour or whether they are due to unequal sister-strand crossing-over within a compound locus it might be expected that one of the mutant spore pairs will differ from the other two with respect to the rest of the chromosome and will resemble the wild type. Cultures from three such *rubiginosa* spore

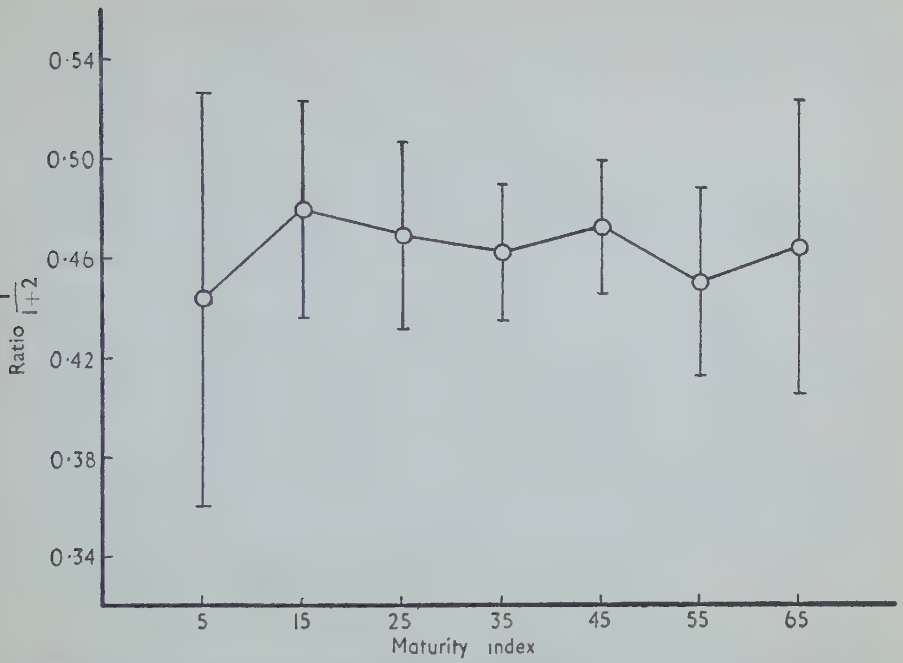

FIG. 1. The relationship between the maturity index of the perithecium and the ratio Class 1/Classes 1 and 2 with confidence limits at the 5 per cent. level of probability.

pairs were used as males and back-crossed to wild type with the following results (Table IV).

TABLE IV

Back Crosses on Wild Type with Males from rubiginosa *Spore-pairs*

Spore pair	Ascus class						Total
	1	2	3	4	5	6	
1	142	109	134	122	94	117	718
2	173	159	166	164	140	146	938
3	197	182	183	193	171	166	1092

In all cases there is a deficiency of class 2 although in crosses with spore pairs 2 and 3 the difference between classes 1 and 2 is not significant. In all cases the symmetric second-division segregations are more frequent than the asymmetric.

3. Albino × *Wild Type*

A total of 3,878 asci were classified as follows:

Ascus class	1	2	3	4	5	6
Ascus frequency	1,173	945	449	468	417	426

The data were internally consistent.

The discrepancy between the two first-division segregation classes is greater than in *rubiginosa* × wild type crosses, $\chi^2_1 = 24{\cdot}5439$ and $P = < 0{\cdot}01$. The four second-division classes, however, do not differ significantly from equality, although the symmetric arrangements are in excess of the asymmetric.

DISCUSSION

There are two obvious sources from which bias may arise in such material. Firstly, it may be introduced by the observer in his method of collecting the data. Measures have been taken to eliminate such sources of error as far as possible.

Secondly the bias may actually exist within the material and may develop either after meiosis or during meiosis. If the former then deviation from equality of the two first-division segregation classes and likewise of the four second-division segregation classes could result from differences in rate of maturing of different arrangements. If this were so it might be expected that the deviation from equality would diminish as the perithecium aged, but no such effect was observed. A similar bias might be introduced by differences in rates of breakdown of asci with different arrangements, but if this were so there should be no deviation from equality in those perithecia where no asci have dehisced.

If the bias occurred during meiosis as a result of differences between homologous chromosomes responding to a gradient in the cytoplasm of the ascus, then in view of the behaviour of the nuclei during their multiplication in the ascus it seems unlikely that there should be any deviation from equality amongst the second-division segregation classes. Furthermore it might be expected that such biasing material should be absent in at least one of the *rubiginosa* spore pairs in abnormal 3 : 1 asci. Back-crosses of three such *rubiginosa* spore pairs to wild type do not support this idea.

The only other tetrad data of any extent classified in this way are those given for *Chromocrea* (Mathieson, 1952). A total of 2,161 asci were classified for segregation of spore size and there is no significant deviation from equality of the six classes; in fact, classes 1 and 2 contain 375 and 379 asci respectively. However, in a set of 512 asci classified for segregation of *albino*, a mutant affecting the colour of the spores, the discrepancy between classes 1 and 2, although not significant, is considerable—108 in class 1 and 85 in class 2. The second-division segregation classes do not differ significantly from equality. It may be significant that in those cases where segregation does appear to be polarized, i.e. *rubiginosa* and *albino* × wild type in *Bombardia* and *albino* × wild type in *Chromocrea*, the mutants in all cases affect the viability or the rate of maturing of the spores. In *Bombardia*, *albino* spores give a lower percentage germination than wild type or *rubiginosa* which in turn is lower than wild type. Whether this is due to differences in rate of maturing or to inviability is not known; probably a combination of both effects, but the polarization observed increases with the decrease in percentage germination. Likewise in *Chromocrea* where *albino* is less viable than wild type, and segregation in the two first-division classes differs from equality, while with spore size, which appears to be neutral in its effect on viability, there is no difference.

How such physiological differences could give rise to apparent biased segregation is not clear. The spores at the apex of the ascus are the first to mature and it may be that unless these apical spores are at a definite stage in relation to conditions in the ascus, the whole ascus fails to mature in a certain proportion of cases. This might account for the discrepancy between classes 1 and 2, but if the effect was sufficient to influence the second-division segregation classes then a deficiency in classes 4 and 5 might be expected and this is not found.

In conclusion it may be said that there is a significant deviation from equality within the two first-division segregation classes and also within the four second-division segregation classes of asci, and that it is unlikely this deviation is due to bias introduced by the observer.

If then the bias is inherent in the material the data suggest that segregation is regular and the bias is the result of physiological differences between whole asci resulting from particular segregations of the genes controlling colour. The reversal of the relative frequencies of asymmetric and symmetric arrangements in Zickler's data is particularly difficult to explain.

SUMMARY

Cultures of wild type and *rubiginosa* strains of Zickler's *Bombardia lunata* were obtained from Germany and an albino mutant subsequently arose spontaneously from the *rubiginosa* strain. The mycelium, perithecia, and ascospores of the wild-type strains are dark olive-green, while in *rubiginosa* strains they are reddish-buff and colourless in the albino.

The *albino* gene is epistatic to the *rubiginosa* and they show 45·2 per cent. recombination.

There is no evidence of linkage between mating type and *rubiginosa* as reported by Zickler.

The behaviour of the nuclei after the second meiotic division in the ascus is such that the plane of the second division is not preserved.

The six ascospore arrangements in asci segregating for *rubiginosa* were scored in 13,487 asci. There is a significant deviation from equality in classes 1 and 2 which is in agreement with Zickler's data although the discrepancy is not so great. In the second-division segregation classes, however, the symmetric classes are significantly greater than the asymmetric classes, whereas in Zickler's data the reverse is found.

Precautions were taken to eliminate bias introduced by the observer and there is no significant difference in the frequencies of the six classes between perithecia of different ages.

Abnormal asci containing three wild-type spore pairs and one *rubiginosa* spore pair and vice versa, provide a means for testing whether chromosomal differences are involved in the polarization. Crosses of cultures from three

such *rubiginosa* spore pairs to wild type gave ascospore arrangements which were not significantly different from each other.

The six ascospore arrangements were also scored in 3,878 asci segregating for *albino*. There is a greater deficiency of class 2 asci compared with class 1 than in the *rubiginosa* crosses, and although the symmetric arrangements in the second-division classes are greater than the asymmetric, the difference is not significant.

There is no obvious explanation for the observed polarized segregation but the evidence suggests that the apparent polarization occurs after meiosis and the effect is a physiological one resulting from particular arrangements of spores, and may be related to the effect on viability of the mutant genes concerned.

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A Rapid Cellulose Peel Technique in Palaeobotany

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ABSTRACT

A quick and easy procedure, employing preformed film, has been devised for the preparation of cellulose peels of petrified plant material.

THE widely used method described by Walton (1928) for the preparation of sections of fossil petrifications involves the formation, on the surface of an etched fossil, of a film from a solution of a cellulose ester. A technique has now been developed in which a preformed film is employed, so that no long drying period is necessary. A very thin cellulose acetate film is used in conjunction with a small quantity of solvent, sufficient only to dissolve the film adjacent to the fossil; the film can be easily pulled off when most of the solvent has evaporated. The present method has been developed from the somewhat similar technique of Sternberg and Belding (1942) for the preparation of fossil animal material, but differs very considerably in the thickness of the peels produced.

The new procedure has several advantages over the original Walton method. There is a great saving in time, especially when it is necessary to obtain many serial sections. The materials are cheap and readily obtainable and no preparation of elaborate solutions is needed. No levelling apparatus is required and there is no trouble from solutions running off etched surfaces. Furthermore, the peels are of constant thickness and can be more easily taken from a fissured and cracked matrix than when solutions of cellulose esters are employed.

Cellulose nitrate film, prepared in the laboratory in the early stages of development of the new method, was as effective as cellulose acetate film, but the latter is preferable since it is more readily obtainable commercially and in addition has the advantages outlined by Walton (1952).

DETAILS OF PROCEDURE

The block of fossil material is ground and etched in the usual way (Lacey, 1953). Grinding is conveniently carried out on sand-blasted glass plates, at first with grade 300 emery powder (and water), the final polishing being with grade 600 emery or emery flour. Careful washing between grindings with different grades is necessary. The block is then etched in hydrochloric or

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hydrofluoric acid, the nature and concentration of the acid and the etching time depending on the material. The acid is washed off by a gentle stream of water, and the etched face dried. Drying is accelerated if the surface is rinsed with 95 per cent. alcohol.

The film is applied to the etched face without pressure. Cellulose acetate film 0·05 mm. thick is highly satisfactory. The etched surface is placed nearly horizontal and a few drops of acetone are added, sufficient to moisten the surface and to form a small pool at one edge. A piece of film of appropriate size is gently laid across the surface of the block, being first applied to the pool of acetone so that no bubbles are trapped. An overlap of film at one side is an advantage, since it facilitates subsequent peeling. If too much fluid remains beneath the film, it may be quickly and gently smoothed down with the finger. The preparation is then set aside to dry; once the film has settled in position it is not advisable to touch the surface, which may soon become tacky. If excess of acetone is used, the film may wrinkle a little but flattens out as the solvent evaporates. Drying is nearly complete in 10–20 minutes. Indeed, excellent peels have been prepared in as little as 7 minutes total time (including etching), but it is best to leave the preparation for an hour or more so that subsequent shrinkage is reduced to a minimum.

The film with its embedded fossil material is peeled off as in the original Walton method. It is advisable to loosen all sides of the peel from the rock—either by gently pulling the projecting edge of the film or by easing it with a razor-blade or scalpel—before removing the peel with a continuous firm pull. The peel may now be trimmed, numbered (Indian ink), and either mounted in Canada balsam or stored unmounted (e.g. between the pages of a book).

TABLE I

Contraction of Films after Drying for various Times at 16° C. and 34° C.

Drying time before peeling	% linear contraction after					
	5 min.	30 min.	2 hr.	2 days	2 weeks	9 weeks
A. At room temp. (16° C.)						
5 min.	1·3	2·4	3·2	3·4	3·4	3·5
15 min.	1·3	2·0	2·7	3·2	3·2	3·3
30 min.	0·9	1·5	2·0	3·0	3·0	3·1
1 hr.	0·7	1·1	1·5	2·4	2·4	2·4
2 hr.	<0·1	0·2	0·4	0·7	0·8	0·8
B. At 34° C.						
15 min.	<0·1	0·2	—	1·3	1·3	1·3
30 min.	<0·1	<0·1	—	0·7	0·7	0·7

CONTRACTION OF FILMS

Unmounted films contract by various amounts depending upon the time and temperature of drying before peeling; subsequent changes in atmospheric conditions have only small effects on the peel. Some results are given in Table I.

Most of the contraction takes place rapidly within the first 48 hours after peeling and thereafter continues very slowly. With peels taken after drying for about 2 hours at room temperature or 30 minutes at 34° C. shrinkage is unlikely to exceed 1 per cent., so that small measurements such as microspore size and cell wall thickness are not significantly affected. Unmounted peels prepared 8 years ago by one of us (W. S. L.) by the Walton method show 4·0–4·5 per cent. contraction while mounted peels of the same age have contracted to a lesser extent (2·0–2·5 per cent.), presumably because the rate of solvent loss is reduced by enclosure in Canada balsam.

Preparations made by the new method, if mounted soon after peeling, thus promise to have the added advantage of less contraction, but, as a safeguard, the original dimensions of the fossil in the rock should always be stated (Walton, 1952).

Scope of Technique

The method has been successfully used on Devonian (siliceous) and Upper Carboniferous (coal ball) material (W. S. L.) and on Lower Carboniferous (calciferous sandstone) material (K. W. J.); the quality of the peels from these various petrifactions compares favourably with that of peels prepared from the same specimens by the older method. No difficulty was experienced in taking complete peels from the largest material available (30 sq. cm.).

The most convenient thickness of cellulose acetate film was found to be 0·05 mm. Thinner peels may be taken but tear readily, and peels of 0·075 mm. or more are inconveniently thick to mount. Films which have proved satisfactory are 'Clarifoil' (British Celanese Ltd., Celanese House, Hanover Square, London, W. 1) and 'Acelon' L. 10 (M & B Plastics Ltd., 45/47 Wigmore Street, London, W. 1).

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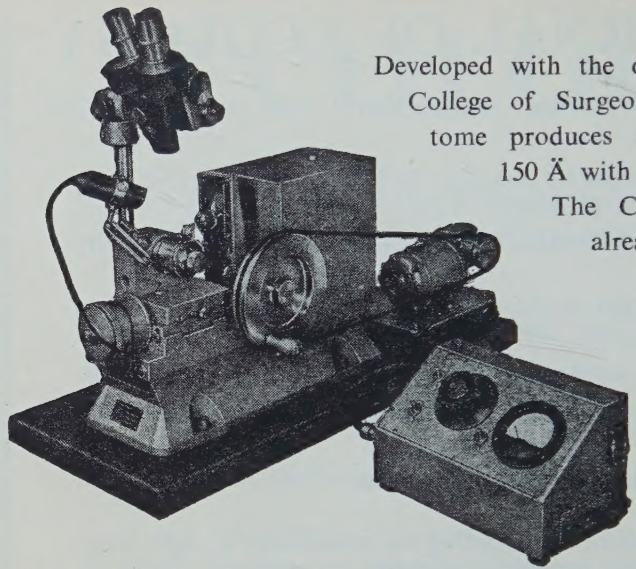

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